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Gender differences in a mouse model of chemotherapy-induced neuropathic pain

Différences selon le sexe des souris dans la douleur neuropathique provoquée par la chimiothérapie

Résumé
La douleur neuropathique provoquée par la chimiothérapie est l’un des principaux problèmes observés chez les patients atteints de cancer. Or, bien que le paclitaxel et la cisplatine soient couramment utilisés chez les femmes, la plupart des études en laboratoire sur la douleur neuropathique provoquée par la chimiothérapie ont été menées sur des animaux mâles. La présente étude a donc examiné chez les souris les différences observées selon le sexe dans la douleur neuropathique consécutive à la chimiothérapie. Les douleurs neuropathiques ont été provoquées par une injection intrapéritonéale de paclitaxel (2 mg/kg) pendant cinq jours consécutifs, et de cisplatine (1 mg/kg) pendant sept jours consécutifs. L’allodynie au froid a été évaluée en mesurant la fréquence de rétraction de la patte et la durée de léchage de la patte par les souris; l’allodynie mécanique a en revanche été mesurée à l’aide des filaments de von Frey. Les douleurs neuropathiques sont apparues au bout de quelques jours (P < 0,001). L’alldynie au froid était plus prononcée chez les souris femelles (P < 0,001) traitées au paclitaxel, tandis qu’aucune différence entre les deux sexes n’a été observée concernant l’allodynie mécanique provoquée par l’administration du paclitaxel. Fait intéressant, aucune différence entre les deux sexes n’a été observée lors des tests relatifs aux allodynies mécaniques et au froid provoquées par l’administration de cisplatine. En conclusion, le sexe des souris joue un rôle majeur dans l’apparition des douleurs neuropathiques consécutives à la prise de paclitaxel. Les différences observées entre les mâles et les femelles devront être prises en compte dans les prochaines études et les résultats devront être généralisés à l’ensemble de la population humaine avec prudence.

Mots-clés
sex, chimiothérapie, douleur neuropathique

Geschlechtsunterschiede bei einem Mausmodell für Chemotherapie-bedingten neuropathischen Schmerz

Abstract

Stichwörter
Geschlecht, Chemotherapie, neuropathischer Schmerz
Diferencias de sexo en modelos de ratón de dolor neuropático inducido por quimioterapia

Resumen
El dolor neuropático inducido por quimioterapia es uno de los principales problemas de los enfermos de cáncer. A pesar de que paclitaxel y cisplatino se utilizan de forma generalizada en mujeres, la mayoría de estudios de laboratorio sobre el dolor neuropático inducido por la quimioterapia se han llevado a cabo en animales machos. El presente estudio examinó las diferencias de sexo en el dolor neuropático inducido por la quimioterapia en ratones. El dolor neuropático fue inducido por una inyección intraperitoneal de paclitaxel (2 mg/kg) durante cinco días consecutivos, y cisplatino (1 mg/kg) durante siete días consecutivos. La alodinia fría fue evaluada midiendo la frecuencia de retirada de la pata y la duración de los lametones de las patas en los ratones; no obstante, la alodinia mecánica fue evaluada por filamentos von Frey. El dolor neuropático empezó a manifestarse transcurridos unos días (P < 0,001). La alodinia fría fue más robusta en los ratones hembra (P < 0,001) tratados con paclitaxel, mientras que no se observaron diferencias entre los dos sexos en la manifestación de alodinia mecánica inducida por paclitaxel. Cabe destacar, que no se observaron diferencias entre sexos en las pruebas de alodinia mecánica y fría inducida por cisplatino. En conclusión, las diferencias entre sexos tienen un papel fundamental en el dolor neuropático inducido por paclitaxel. Las diferencias entre animales macho y hembra deberían tenerse en cuenta en estudios futuros y las conclusiones deberían generalizarse en humanos con precaución.

Palabras clave
sexo, quimioterapia, dolor neuropático

Effects of type of light on mouse circadian behaviour and stress levels

Effets des différents types de lumière sur les rythmes circadiens et les niveaux de stress chez la souris

Résumé
La lumière représente le principal facteur environnemental de la synchronisation de l'horloge biologique. La quantité de lumière (l'intensité) et la qualité de la lumière (le type de source lumineuse) peuvent produire différents effets. L'objectif de cette étude consistait à déterminer les effets des différents types de lumière rencontrés par les souris depuis leur naissance sur leur développement, leurs rythmes circadiens et leurs niveaux de stress. Pendant la période d'allaitement, des souris pigmentées et albinos ont été soumises à des cycles jour/nuit de 24 heures présentant une source lumineuse fluorescente ou à diodes électroluminescentes (LED) blanches, et ont ensuite été exposées à différentes conditions d'éclairage après leur sevrage, avant que soient mesurés leur taux de croissance, leur activité locomotrice et leurs taux plasmatiques de corticostérone. Il a été découvert que le type de lumière auquel étaient exposées les souris n'avait aucune incidence sur leur taux de croissance ou leurs niveaux de stress. Des effets importants ont toutefois été observés sur les rythmes de l'activité locomotrice des souris pigmentées au cours des cycles jour/nuit faiblement contrastés, mais aussi chez les souris albinos et pigmentées soumises à un éclairage permanent. Ces résultats soulignent l'importance de la qualité de l'environnement lumineux (la source de lumière) pour les rythmes circadiens et la nécessité d'un suivi étroit des environnements lumineux au sein des animaleries.

Mots-clés
souris, albinos, pigmenté, lumière fluorescente, lumière LED

Auswirkungen von Beleuchtungsarten auf zirkadianes Verhalten und Stressniveau von Mäusen

Abstract
sowohl bei Albino- als auch bei pigmentierten Mausen. Diese Ergebnisse unterstreichen die Bedeutung der Lichtqualität der Umgebung (Lichtquelle) für zirkadiane Verhaltensrhythmen sowie die Notwendigkeit einer strengen Überwachung der Lichtbedingungen in Tiereinrichtungen.

Stichwörter
Maus, Albino, pigmentiert, Neonlicht, LED-Beleuchtung

Efectos del tipo de luz en el comportamiento circadiano y en los niveles de estrés de ratones

Resumen
La luz es el principal factor medioambiental sincronizador para el reloj biológico. La cantidad de luz (intensidad) y la calidad de luz (tipo de fuente de luz) pueden tener efectos distintos. El objetivo del presente estudio fue determinar los efectos del tipo de luz experimentados desde el momento del nacimiento en el crecimiento del ratón, el comportamiento circadiano y los niveles de estrés. Criamos ratones albinos y pigmentados en ciclos de 24 h luz-oscuridad con fuentes de luz blanca con diodo emisor de luz (LED) o fluorescentes durante la fase lactante, luego se expusieron a los animales a varios entornos lumínicos después del destete y se midió su ritmo de crecimiento, actividad locomotora y la concentración de corticosterona plasmática. Encontramos que el tipo de luz al que se exponían a los animales no tuvo ningún efecto en el ritmo de crecimiento y en los niveles de estrés de los mismos. No obstante, observamos efectos significativos en la expresión del ritmo de la actividad locomotora bajo ciclos de contraste luz-oscuridad en ratones pigmentados, y bajo luz constante en ratones pigmentados y albinos. Estos resultados destacan la importancia de la calidad de luz medioambiental (fuente de luz) en los ritmos de comportamiento circadiano, y la necesidad de llevar a cabo un control exhaustivo de los entornos de luz en las instalaciones para animales.

Palabras clave
ratón, albino, pigmentado, luz fluorescente, luz LED

Assessing the welfare of laboratory mice in their home environment using animal based measures – a benchmarking tool


Évaluer le bien-être des souris de laboratoire dans leur environnement habituel au moyen de mesures axées sur les animaux comme outils d’évaluation comparative

Résumé
Les problèmes de bien-être des souris de laboratoire peuvent être la conséquence d’une expérimentation en cours ou la caractéristique d’une lignée génétique particulière, mais dans certains cas, par exemple chez les animaux reproducteurs, ils sont le plus souvent liés à l’agencement et à l’entretien des cages. L’évaluation de l’environnement des cages est fréquemment réalisée au moyen de mesures reposant sur des ressources, par exemple l’accès aux matériaux de nidification. Néanmoins, les mesures axées sur les animaux (qui sont liées à l’état de santé et au comportement de ces derniers) peuvent être utilisées pour évaluer le bien-être réel des animaux quelles que soient les données utilisées [ex: les ressources ou leur gestion]. L’objectif de cette étude visait à concevoir un protocole permettant d’évaluer le bien-être des souris de laboratoire en utilisant uniquement des mesures axées sur les animaux. Ce protocole, qui doit être utilisé comme un outil d’évaluation comparative, analyse le bien-être des souris au sein des cages et ne contient aucun paramètre lié à des situations expérimentales. Il est fondé sur les paramètres correspondant aux 12 critères établis en matière de bien-être dans le cadre du projet Welfare Quality®. Le choix des mesures axées sur les animaux a été effectué en passant en revue les protocoles informels, ceux ayant fait l’objet d’une publication et ceux disponibles sur la Toile, et en sélectionnant des paramètres répondant à ces critères, applicables dans la pratique et, si possible, qui constituaient déjà des indicateurs validés en matière de bien-être des souris. Ces paramètres doivent permettre d’identifier d’éventuels problèmes liés au bien-être des animaux et de réaliser une évaluation directement dans les salles où ils sont installés, au cours du processus de nettoyage des cages, et sans devoir faire usage d’équipements supplémentaires. Les comportements liés au confort thermique et les états émotionnels positifs sont des domaines dans lesquels il serait nécessaire d’effectuer des travaux de recherche supplémentaires afin d’identifier des mesures axées sur les animaux qui soient valables, fiables et concrètes.

Mots-clés
bien-être des animaux de laboratoire, évaluation du bien-être, logement, élevage, techniques de manipulation, éducation

Translated abstracts

sowohl bei Albino- als auch bei pigmentierten Mausen. Diese Ergebnisse unterstreichen die Bedeutung der Lichtqualität der Umgebung (Lichtquelle) für zirkadiane Verhaltensrhythmen sowie die Notwendigkeit einer strengen Überwachung der Lichtbedingungen in Tiereinrichtungen.

Stichwörter
Maus, Albino, pigmentiert, Neonlicht, LED-Beleuchtung

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Palabras clave
ratón, albino, pigmentado, luz fluorescente, luz LED

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Mots-clés
bien-être des animaux de laboratoire, évaluation du bien-être, logement, élevage, techniques de manipulation, éducation
Bewertung von tiergerechter Haltung und Wohlbefinden von Versuchsmäusen in ihrer heimischen Umgebung mittels tierbezogener Indikatoren – ein Benchmarking-Instrument

Abstract

Stichwörter
Wohlbefinden von Versuchstieren, Tiergerechtheitsbewertung, Unterbringung, Tierhaltung, Verfahren, Vervollkommnung

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Evaluación del bienestar de los ratones de laboratorio en su entorno de alojamiento utilizando mediciones basadas en animales: una herramienta de referencia

Resumen
Los problemas de bienestar en ratones de laboratorio pueden ser una consecuencia de un experimento en curso, o una característica de una línea genética en particular, pero en algunos casos, como en animales de cría, es más probable que sean el resultado del diseño y la gestión de la jaula de alojamiento. Normalmente se lleva a cabo una evaluación del entorno de la jaula de alojamiento utilizando medidas basadas en recursos, como acceso a material de anidamiento. No obstante, pueden utilizarse medidas basadas en animales (en relación al estado de salud y al comportamiento de los animales) para evaluar el bienestar actual de los animales independientemente de las aportaciones aplicadas (es decir, los recursos o gestión). El objetivo de este estudio fue diseñar un protocolo para evaluar el bienestar de ratones de laboratorio utilizando únicamente medidas basadas en animales. El protocolo, a ser utilizado como una herramienta de referencia, evalúa el bienestar de los ratones en la jaula de alojamiento y no contiene parámetros relacionados con situaciones experimentales. Se basa en parámetros correspondientes a los 12 criterios de bienestar establecidos por el proyecto Welfare Quality®. Se llevó a cabo una selección de medidas basadas en animales analizando protocolos informales, en Internet y publicados, y eligiendo parámetros que correspondieran a estos criterios, que fueran factibles y, en la medida de lo posible, que ya fueran indicadores válidos del bienestar de los ratones. Los parámetros debían identificar posibles problemas de bienestar en animales y permitir una evaluación directa en salas de animales durante los procedimientos de limpieza de jaulas, sin la necesidad de equipos adicionales. Los comportamientos de confort termal y estados emocionales positivos son áreas que requieren más investigación para encontrar medidas basadas en animales que sean factibles, fiables y válidas.

Palabras clave
bienestar de animales de laboratorio, evaluación de bienestar, alojamiento, cría de animales, técnicas de manipulación, refinamiento
The effect of Syphacia muris on nutrient digestibility in laboratory rats


Effet du parasite intestinal *Syphacia muris* sur la digestibilité des aliments chez les rats

Résumé

Mots-clés
rat de laboratoire, *Syphacia muris*, infection, aliment, digestibilité

Auswirkungen von *Syphacia muris* auf Nährstoffverdaulichkeit bei Laborratten

Abstract

Stichwörter
Laborratte, *Syphacia muris*, Infektion, Nährstoff, Verdaulichkeit

El efecto de *Sifacia muris* en la digestibilidad de nutrientes en ratas de laboratorio

Resumen
El presente estudio se llevó a cabo para investigar cómo la infección de oxiuros en ratas afecta a la digestibilidad de nutrientes en los huéspedes. Veinticuatro ratas Wistar macho no consanguíneas fueron divididas aleatoriamente en dos grupos de 12 ratas cada uno. Las ratas del primer grupo (GI) se introdujeron en jaulas con nidos con huevos de oxiuros, y el segundo grupo (control) (GII) se introdujo en una sala separada y limpia con jaulas con la parte superior de filtro, y sin contaminar. Los animales fueron introducidos posteriormente en jaulas individuales metabólicas. Las pruebas metabólicas duraron cinco días y se tomaron registros diarios del peso de los animales, la ingesta de comida y el peso de las heces. Basándose en análisis de laboratorio del contenido de nutrientes en los alimentos y las heces, se determinó los valores de digestibilidad. El día 15 del experimento, los animales se sometieron a eutanasia. A pesar de que se encontró *Sifacia muris* en todas las ratas del grupo GI, los animales no mostraron ningún signo clínico. En nuestro experimento, la
infección de S. muris redujo la digestibilidad general de todos los nutrientes medidos (P < 0,01). Las diferencias más significativas en la digestibilidad se observaron en el caso de fibra de crudo y materia mineral (P < 0,01).

Palabras clave
rata de laboratorio, *Sifacia muris*, infección, nutriente, digestibilidad

Effective cryopreservation of golden Syrian hamster embryos by open pulled straw vitrification


Cryoconservation efficace des embryons d’hamsters dorés syriens au moyen de la technique de vitrification rapide à l’aide de paillettes étirées

Résumé
Il est difficile de procéder à la cryoconservation des embryons d’hamsters dorés syriens en raison de leur sensibilité élevée aux cryoprotecteurs et à la manipulation *in vitro*. L’objectif de cette étude consiste à développer une technique robuste de vitrification rapide utilisant des paillettes étirées (OPS) permettant de cryoconserver des embryons d’hamsters à différents stades de leur développement. Il a d’abord été procédé au test systématique des concentrations de cryoprotecteurs et des temps d’exposition d’embryons à deux cellules à différentes solutions de vitrification. Le prétraitement des embryons à deux cellules suivant a été identifié comme étant le meilleur protocole de vitrification rapide à l’aide de paillettes étirées (OPS): 10% (v/v) d’éthylène glycol (EG) +10% (v/v) de diméthylsulfîde-oxide (DMSO) pendant 30 secondes, puis une exposition dans la solution de vitrification, EDFS30 (contenant 15% d’EG et 15% de DMSO), pendant 30 secondes, avant de placer les embryons dans de l’azote liquide (méthode d’exposition comprenant deux étapes). Les ressources offertes par ce protocole permettant de vitrifier des embryons d’hamsters à différents stades de leur développement ont ensuite été étudiées. Les résultats ont indiqué que ce protocole permettait d’obtenir des taux de blastocystes élevés à partir d’embryons vitrifiés au cours des stades à deux cellules, quatre cellules, huit cellules ou au cours du stade morula (62%, 78%, 80% ou 72%, respectivement), mais pas à partir des cellules qui ont été vérifiés aux stades pro-nucléaire (0%) et de blastocystes (24%; P < 0,05). Lorsque les embryons vitrifiés au cours du stade à deux cellules furent récupérés puis directement transférés dans des femelles receveuses, 29% d’entre eux se sont développés jusqu’au terme de la grossesse, un taux de développement qui ne varie pas significativement (P > 0,05) des 40% représentant le taux de natalité au sein des groupes témoin non vitrifiés. En conclusion, il a été élaboré pour les embryons d’hamsters un protocole efficace de vitrification rapide en deux étapes utilisant des paillettes étirées.

Mots-clés
hamster, embryon, vitrification rapide à l’aide de paillettes étirées [OPS]

Effektive Kryokonservierung von Embryonen syrischer Goldhamster durch Open Pulled Straw-Vitrifikation

Abstract
Die Kryokonservierung syrischer Goldhamsterembryonen ist aufgrund ihrer hohen Empfindlichkeit gegenüber Gefrierschutzmitteln und einer *In-Vitro*-Behandlung schwierig. Ziel dieser Studie war die Entwicklung eines robusten OPS-Verfahrens zur Kryokonservierung von Hamsterembryonen in unterschiedlichen Entwicklungsstadien. Wir testeten zunächst systematisch die Konzentrationen von Gefrierschutzmitteln und die Kontaktzeiten zweizelliger Embryos mit verschiedenen Vitrifikationslösungen. Als optimales OPS-Vitrifikations-Protokoll identifizierten wir die Vorbehandlung zweizelliger Embryos mit 10% (v/v) Ethylenglycol (EG) +10% (v/v) Dimethylsulfîde-oxide (DMSO) für 30 s, anschließendes Einbringen in die Vitrifikationslösung EDFS30 (bestehend aus 15% EGp15% DMSO) für 30 s und anschließendes Einthauchen in flüssigen Stickstoff [Zwei-Schritt-Expositionsmodell]. Wir untersuchten sodann die Robustheit dieses Protokolls für die Vitrifikation von Hamsterembryos in unterschiedlichen Entwicklungsstadien. Die Ergebnisse zeigten, dass dieses Protokoll hohe Blastozysten-Quoten von Embryos erzielt, die im zweizelligen, vierzelligen, achttzelligen oder Morula-Stadium (62%, 78%, 80% bzw. 72%) eingefroren wurden, nicht jedoch von solchen, die im pronuklearen (0%) oder Blastozysten-Stadium vitrifiert wurden (24%; P < 0,05). Nach dem Auftauen von im Zweizellen-Stadium eingefrorenen Embryonen und ihrem unmittelbar darauf folgenden Transfer zu empfangenden Weibchen entwickelten sich 29% bis zur Austragung, eine Entwicklungsquote, die sich nicht wesentlich (P > 0,05) von der 40%igen Geburtsquote der nicht eingefrorenen Kontrollgruppe unterschied. Daraus ergibt sich, dass unsere Methode ein effektives Zwei-Schritte-OPS-Vitrifikationsprotokoll für Hamsterembryonen darstellt.

Stichwörter
Hamster, Embryo, OPS-Vitrifikation
Criopreservación eficaz de embriones de hámster dorado sirio mediante vitrificación open pulled straw

Resumen
Los embriones de hámster dorado sirio son difíciles de criopreservar debido a su alta sensibilidad a los crioprotectores y a la manipulación in vitro. El objetivo del presente estudio es desarrollar una técnica de vitrificación open pulled straw (OPS) para criopreservar embriones de hámster en varias fases de desarrollo. En primer lugar, probamos sistemáticamente las concentraciones de crioprotectores y los tiempos de exposición de embriones de dos células a varias soluciones de vitrificación. Identificamos un pretratamiento de embriones de dos células con un 10% (v/v) de etilenglicol (EG) + 10% (v/v) dimetilsulfóxido (DMSO) durante 30 s seguido de una inmersión en la solución de vitrificación, EDFS30 (que contiene 15% EG y 15% DMSO), durante 30 s antes de sumergir en nitrógeno líquido (método de exposición en dos pasos) como el protocolo de vitrificación OPS óptimo. A continuación investigamos la iniciativa de este protocolo para vitrificar embriones de hámster en distintas fases de desarrollo. Los resultados demostraron que con este protocolo se consiguieron unos altos índices de blastocistos de embriones vitrificados en dos células, cuatro células, ocho células, o estado de mórlula (62%, 78%, 80% o 72% respectivamente), pero no los vitrificados en la fase pronuclear (0%) o de bastocisto (24%; P < 0,05). Cuando se recuperaban embriones vitrificados en la fase de dos células y luego se transferían directamente a hembras receptores, un 29% de éstas desarrollaron un índice de desarrollo no significativamente distinto (P > 0,05) al del 40% del índice de nacimiento de los controles no vitrificados. En conclusión, hemos creado un protocolo de vitrificación OPS de dos fases para embriones de hámster.

Palabras clave
hámster, embrión, vitrificación OPS

Refinement of a thermal threshold probe to prevent burns


Amélioration d’une sonde de seuils thermiques visant à éviter les brûlures

Résumé
Les essais liés aux seuils thermiques sont couramment utilisés pour les travaux de recherche sur la douleur. Le stimulus peut entraîner des brûlures et mérite une stratégie de prévention. Des modifications de sondes thermiques supposées réduire les brûlures ont été évaluées en termes de fonctionnalité et d’efficacité. Les études ont été réalisées sur deux personnes et huit chats. La sonde non modifiée a été testée sur deux personnes et les modifications prometteuses ont également été testées sur les chats. La sonde 1 offrait un refroidissement rapide une fois le seuil atteint: la sonde 1a était munie d’un système Peltier et la sonde 1b d’un système de refroidissement à eau. La sonde 2 a permis d’éviter immédiatement tout contact avec la peau une fois le seuil atteint. La sonde 3 a été développée suite à l’identification de « points chauds » dans la sonde 0l occasionnées une réduction de la masse thermique et un chauffage uniforme sur la surface de contact avec la peau. La peau humaine a été chauffée à une température de 48°C (6°C au-dessus du seuil) et la brûlure occasionnée a été évaluée sur la base de la zone affectée et d’une simple échelle descriptive (SED). La sonde 1a a permis de refroidir la peau mais n’a nécessité une dissipation additionnelle de chaleur et une utilisation excessive d’énergie. Par ailleurs, elle ne constituait pas un appareil éprouvé et ne pouvait pas être installée sur des animaux. La sonde 1b a provoqué moins de dommages qu’une sonde n’offrant aucun refroidissement (27±13 et 38±11mm² respectivement, P = 0,0266; valeur médiane de la SED: 1,5 et 4 respectivement, P = 0,0317), mais elle était encombrante. La sonde 2 était peu maniable et n’a pas été étudiée davantage. La sonde 3 a offert un chauffage uniforme et n’a engendré aucune cloque chez les personnes l’ayant utilisée. Lorsque la sonde 3 a été utilisée sur les chats, après le traitement aux opioïdes, le seuil thermique a été atteint [55°C] 24 fois, une température supérieure à 50°C a été atteinte lors de 32 tests supplémentaires et une température supérieure à 48°C a été atteinte au cours de tous les autres tests. Aucune lésion cutanée n’était visible immédiatement après les tests et une légère hyperémie observée chez trois chats au bout de 2–3 jours a rapidement disparu. La sonde 3 s’est révélée adéquate pour les tests liés aux seuils thermiques.

Mots-clés
amélioration, douleur, seuil neuroceptif, chat, thermique
Vervollkommnung einer Wärmeschwellwert-Sonde zur Prävention von Verbrennungen

Abstract
Das Testen thermischer Schwellenwerte findet in der Schmerzforschung häufig Anwendung. Der thermische Reiz kann Verbrennungen verursachen, deren Prävention anzustreben ist. Modifizierte Wärmeschwellensonden, deren Fähigkeit zur Reduzierung von Verbrennungen angenommen wird, wurden auf Zweckmäßigkei und Wirkung untersucht. Dazu fanden Studien mit zwei Menschen und acht Katzen statt. Eine nicht modifizierte Sonde 0 wurde bei zwei Menschen getestet, zudem wurden vielversprechende Modifizierungen auch bei Katzen untersucht. Sonde 1 bot schnelle Abkühlung nach Erreichen des Schwellenwertes: Sonde 1a basierte auf einem Peltier-System und Sonde 1b auf Wasserkühlung. Bei Sonde 2 wurde unmittelbar nach Schwellenwerterreichung der Hautkontakt aufgehoben. Sonde 3 (entwickelt mit Blick auf in Sonde 0 festgestellte „Hotspots“) reduzierte thermische Masse und sogar Hitze über die Hautkontaktfläche hinweg. Menschliche Haut wurde auf $48^\circ\text{C}$ ($6^\circ\text{C}$ über Schwellenwert) erhitzt und die verursachte Verbrennung anhand der Verletzungsfläche und einer einfachen deskriptiven Skala (simple descriptive scale – SDS) beurteilt. Sonde 1a kühlte die Haut, erforderte aber weitere Hitzeableitung und ein Übergewicht an Strom, war nicht ausfallsicher und für die Anbringung an Tieren ungeeignet. Sonde 1b verursachte weniger Schaden als keine Kühlung ($27^\circ\text{C}$ und $38^\circ\text{C}$ für Sonde 1b, $38^\circ\text{C}$ und $38^\circ\text{C}$ für Sonde 1a, $P = 0.0266$; SDS-Median 1,5 bzw. 4, $P = 0.0317$), war aber umständlich zu handhaben. Sonde 2 war unhandlich und wurde nicht weiter bewertet. Sonde 3 erzeugte beim Menschen gleichmäßige Hitze ohne Blasenbildung. Mit Sonde 3 wurde bei Katzen nach Opioid-Behandlung in 24 Fällen der Wärmeschwellen-Abschaltwert ($55^\circ\text{C}$) erreicht, bei weiteren 32 Tests wurden $50^\circ\text{C}$ und bei den restlichen Tests $48^\circ\text{C}$ überschritten. Hautsäden waren unmittelbar nach den Tests nicht erkennbar, und bei drei Katzen an 2–3 Tagen auftretende milde Hyperämie verschwand schnell. Sonde 3 scheint für Tests thermischer Schwellenwerte geeignet zu sein.

Stichwörter
Vervollkommnung, Schmerz, nozizeptive Schwelle, Katze, thermal, Wärme

Refinamiento de una sonda de límite térmico para evitar quemaduras

Resumen
Normalmente se realizan pruebas de límites térmicos para los estudios de dolor. El estímulo puede causar quemaduras y la prevención de beneficios. Se evaluó la funcionalidad y efecto de las modificaciones de las sondas térmicas planteadas como hipótesis para reducir las quemaduras. Se realizaron estudios en dos humanos y ocho gatos. Una sonda 0 no modificada fue probada en dos humanos y se evaluaron unas modificaciones prometedoras en gatos. La sonda 1 incorporó un enfriamiento rápido después de alcanzar el límite: la sonda 1a utilizó un sistema Peltier y la sonda 1b utilizó un enfriamiento con agua. La sonda 2 suministró un contacto con la piel inmediatamente después de alcanzar el límite. La sonda 3 (desarrollada en función de la evidencia de "puntos calientes" en la sonda 0) incorporó una masa térmica reducida y calor equitativo en toda la zona de contacto dérmica. La piel humana alcanzó una temperatura de $48^\circ\text{C}$ ($6^\circ\text{C}$ por encima del límite) y la quemadura resultante fue evaluada utilizando una zona de lesiones y una simple escala descriptiva (SDS). La sonda 1a enfrió la piel pero requirió una mayor disipación del calor, poder excesivo, no era "a prueba de fallos" y no era apropiada para la monta de animales. La sonda 1b causó menos daños que no enfriamiento ($27^\circ\text{C}$ y $38^\circ\text{C}$ para Sonde 1b, $38^\circ\text{C}$ y $38^\circ\text{C}$ para Sonde 1a, $P = 0.0266$; mediana SDS 1,5y 4, $P = 0.0317$) pero era difícil de manejar. La sonda 2 era poco manejable y no se continuó su evaluación. La sonda 3 produjo un calor equitativo sin abrasiones en humanos. Con la sonda 3 en gatos, después de un tratamiento opioide, el límite térmico alcanzó una disyunción ($55^\circ\text{C}$) en 24 ocasiones, excedió los $50^\circ\text{C}$ en otras 32 pruebas y sobrepasó los $48^\circ\text{C}$ en las restantes. No se observó ningún daño evidente en la piel inmediatamente después de las pruebas y una hiperemia leve en tres gatos en 2–3 días se resolvió rápidamente. La sonda 3 parecía ser idónea para la realización de pruebas de límite térmico.

Palabras clave
refinamiento, dolor, límite nociceptivo, gato, térmico

The effect of anaesthesia on somatosensory evoked potential measurement in a rat model

Effet de l'anesthésie sur les potentiels évoqués somatosensoriels mesurés chez les rats
Résumé
Les potentiels évoqués somatosensoriels (PES) sont couramment utilisés pour étudier l’intégrité fonctionnelle des voies sensorielles ascendantes. Dans le cadre des études animales, les PES constituent une méthode pratique et peu invasive qui permet d’évaluer de
manière quantitative la fonctionnalité du système nerveux. Bien que les PES soient fréquemment utilisés dans les modèles animaux, une attention insuffisante est consacrée au fait qu’ils sont vulnérables à la contamination par des facteurs expérimentaux tels que l'administration d'anesthésiques. Dans le cadre de cette étude, on a analysé chez les rats l’effet de l’isoflurane sur les mesures des PES. L’objectif consistait à identifier les ajustements nécessaires à apporter à l’administration d’anesthésiques en vue d’optimiser la qualité des données obtenues. Deux aspects ont été étudiés : l’effet du dosage d’isoflurane sur les paramètres liés aux PES et sur la reproductibilité des mesures. C’est en utilisant une concentration d’isoflurane d’1,5% que les PES ont présenté le meilleur niveau de qualité. Cette posologie a permis de produire le meilleur rapport signal/bruit et une reproductibilité des mesures équivalente, comparée aux autres dosages. Les conclusions de cette étude peuvent aider à améliorer les protocoles d’anesthésie liés aux mesures des PES chez les rats, et en augmentant la qualité des mesures, elles peuvent potentiellement réduire le nombre de sujets nécessaires pour réaliser des études.

Mots-clés
anesthésie, modèle animal, réduction, rongeurs

Auswirkung von Anästhesie auf Messungen somatosensorisch evozierter Potenziale bei einem Rattenmodell

Abstract

Stichwörter
Anästhesie, Tiermodell, Reduzierung, Nager

El efecto de la anestesia en la medición de potenciales evocados somatosensoriales en modelos de rata

Resumen
Los potenciales evocados somatosensitivos (SEPs) se utilizan ampliamente para estudiar la integridad funcional de vías sensoriales ascendentes. Para estudios con animales, los SSEP representan un método conveniente para evaluar cuantitativamente la funcionalidad del sistema nervioso con baja invasión. A pesar de que se utilizan con frecuencia en modelos de animales, no se presta mucha atención al hecho de que los SSEP son vulnerables a la contaminación de factores experimentales como el suministro anestésico. En este estudio se investigó en una rata el efecto de isoflurano en mediciones SSEP. El objetivo era averiguar los ajustes para el suministro anestésico optimizando la calidad de los registros. Se estudiaron dos aspectos: el efecto de la dosis de isoflurano en los parámetros SSEP y en la repetibilidad de las mediciones. La calidad de los SSEP se encontró que era superior al utilizar una concentración de isoflurano de 1,5%. Esta dosis ofreció el mejor ratio señal/ruido y repetibilidad igual de las mediciones en comparación con otros. Nuestros hallazgos pueden ayudar a refinar los protocolos anestésicos en relación a los registros de SSEP en un modelo de rata y, al mejorar la calidad de las mediciones, reducir potencialmente el número de sujetos necesario para realizar los estudios.

Palabras clave
anestesia, modelo animal, reducción, roedores
Neotropical primate nursery in a squirrel monkey breeding unit in Brazil

Nursery pour primates néotropicaux dans une unité d’élevage de singes écureuils au Brésil
Résumé
Le Saimiri (singe écureuil) est un primate néotropical d’origine simienne qui a été élevé en captivité à des fins de recherche sur la santé humaine et animale. Il est fréquemment utilisé dans les études sur l’ophthalmologie, la toxicologie, la pharmacologie, la psychiatrie, les sciences neurologiques, les tests de vaccins et de médicaments (tels que les agents liés au paludisme et à la rougeole), ainsi que dans les études sur les effets observés sur le comportement interactif et sur les connaissance liées à la maladie de Creutzfeldt-Jakob chez l’homme. Les principaux travaux des centres de recherche sur les primates non humains (PNH) concernent essentiellement l’établissement de colonies reproductives autonomes fournissant des animaux expérimentaux de bonne qualité. Le rejet par la mère, la dystocie et la pneumonie sont les principales causes de décès de nouveau-nés parmi ces espèces. Par conséquent, afin de garantir la survie de ces précieux animaux, le Centre d’élevage d’animaux de laboratoire de la Fondation Oswaldo Cruz (CECAL ou Fiocruz) de Rio de Janeiro, au Brésil, a élaboré un protocole lié à l’élevage de ces petits primates dans des nursery.

Mots-clés
singes écureuils, nursery, élevage, nouveau-nés, élevage à la main

Pflege neotropischer Primaten in einer Totenkopfaffen-Aufzuchtstation in Brasilien
Abstract

Stichwörter
Totenkopfaffe, Pflegestation, Aufzucht, Neugeborene, Jungtiere, Handaufzucht

Crianza de primates neotropicales en una unidad de cría de monos ardilla en Brasil
Resumen
El Saimiri (mono ardilla) es un primate neotropical de género simio que ha sido criado en cautiverio para el desarrollo de estudios de investigación para la salud de humanos y animales. Han sido extensamente utilizados en estudios de oftalmologia, toxicología, farmacología, psiquiatría, neurociencia, vacunas y pruebas de medicamentos (como malaria y agentes de sarampión), además de para estudiar los efectos en el comportamiento interactivo y cognitivo de la enfermedad de Creutzfeldt-Jakob en hombres. La principal preocupación de los centros de investigación de primates no humanos (PNH) se centra en establecer colonias de cría autosostenibles que ofrezcan animales de investigación de buena calidad. El rechazo materno, distocia y la pulmonía son las principales causas de las muertes en los recién nacidos de esta especie. Por tanto, para poder garantizar la supervivencia de estos valiosos animales, el Centro de Cria de Animales de Laboratorio de la Fundación Oswaldo Cruz (CECAL)/Fiocruz, Río de Janeiro, Brasil, ha creado un protocolo para la cría de estos recién nacidos.

Palabras clave
Monos ardilla, crianza, cría, neonatos, cría manual
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Special editorial for the 50th anniversary of Laboratory Animals Limited

The Foundation of Laboratory Animals Limited

Although Laboratory Animals Limited (LAL) is now celebrating its 50th year, the events which lead to its formation took 25 years in the making.

It was as early as 1942 that the Pathological Society of Great Britain proposed to the Medical Research Council (MRC) and the Agricultural Research Council (ARC) that the Society should 'take up the question of large scale breeding of healthy experimental animals as a matter of national importance and urgency'. These Research Councils were not convinced of the efficiency or safety of concentrating the breeding in one or two national centres. A Medical Sciences Committee was inaugurated and held open meetings in 1942 and 1943 and subsequently supported the need for a national scheme for the supply of animals.

A Standing Committee was formed to survey the capacity of existing breeding establishments in the UK and to suggest how to set up a central information service to give them advice on how to improve the quality of the animals in terms of genetics, housing and nutrition.

By June 1944 the Standing Committee had met five times and had received responses from 262 users and 896 breeders and dealers of animals in the UK! It then proposed the formation of an Advisory Committee of users and breeders, with the possibility of a small executive forming a central Bureau. Its only stipulation was that one geneticist should serve, as the establishment and maintenance of 'pure strains' was a primary concern. The Bureau was to collect and act as a clearing house for information on supply, demand and availability of stocks of animals.

It produced a 19-page Memorandum stating its intention to 'provide a bibliographical service on any problem concerning experimental animals or their use for special purposes and publication of a periodical for wide distribution'.

The Laboratory Animals Bureau was established at the MRC National Institute for Medical Research but soon moved to the Royal Veterinary College. Its first Director was Ronald E Glover who would soon be replaced by the inspirational appointment of William (Bill) Lane-Petter. Lane-Petter was qualified in natural science and medicine and had spent three years as a Home Office Inspector of animal experiments under the 1876 Cruelty to Animals Act. With his ability to solve problems and motivate staff, and persuade people in power, the Bureau rapidly made an impact. A legendary story details how he established the Bureau's first colony of white mice (Laboratory Animals Centre A-strain mice [LACA]) by carrying a pair with him in his jacket pocket on the flight back from the USA!

The Bureau became the Laboratory Animals Centre (LAC) and John Bleby took over from Lane-Petter. The world’s first independent Accreditation Scheme for laboratory animal breeders was established. Apart from its main work of improving the quality, care and availability of laboratory animals, the LAC also convened conferences and published their proceedings. The regular ‘News Letter’ and ‘LAC Collected Papers’ (Volume 1 of which came out in 1953) were the de facto predecessors of the journal. They were distributed on request to anyone free of charge.

These peripheral activities eventually posed problems in the justification of resources and by 1963 discussions were under way to set up a society to undertake such work and so the UK Laboratory Animal Science Association (LASA) was established.

From the outset LASA wished to offer some sort of publication as a privilege of membership. An informal agreement was made with an existing journal to ‘look kindly’ on papers submitted to it by LASA members. It was clear, however, that the journal in question was not appropriate for the range of interests to be found in LASA. Peter C Williams was asked to convene a Publications Working Party. The Institute of Animal Technicians (IAT; later Technology) was approached about the possibility of a joint journal and these negotiations were still ongoing when the Working Party reported to Council in February 1966. Eventually the IAT rejected the proposal for a joint journal and the LASA Council invited Williams to become Chairman of an Editorial Board (which was made up of all the members of the Working Party).
At the November 1966 Council, Williams put forward the Editorial Board’s recommendation that it should form from LASA members a Company, limited by guarantee, in order to publish ‘an independent journal devoted to laboratory animal science’. This recommendation was accepted.

At the February 1967 Council meeting, Williams reported that ‘Laboratory Animals Limited’ was in the process of registration as a company and that the first issue of the new journal, Laboratory Animals, was on course for publication on the 2nd of April that year.

At the LASA Annual General Meeting (AGM) of November 1967 the Executive Editor was able to report that a first annual volume of 176 pages of text in two parts had been published and that the first of an occasional series of ‘Laboratory Animal Symposia’ (later to be renamed ‘Laboratory Animal Handbooks’) was in production.

Malcolm R Gamble
former Chairman of Laboratory Animals Limited
(with thanks to Philip N O’Donoghue, former Executive Editor of Laboratory Animals)
Laboratory Animals, the Journal (LAJ, LAN, LA–UK, Lab Anim)

The first issue of Laboratory Animals was published on 1 April 1967. Up to 1971, two issues per year were published, followed by three issues per year. From 1975 up to 2015 four issues appeared annually, with some additional supplements on special topics. Over the past 50 years many outstanding editors contributed with their hard work to the success of the journal. Special thanks go to Philip N O’Donoghue, Paul Flecknell, Timothy Morris and Colin Dunn.

The international journal Laboratory Animals is the official journal of several subscribing societies, AFSTAL, ESLAV, FELASA, GV-SOLAS, ILAF, LASA, NVP, SECAL, SGV and SPCAL. As one can see on the cover, the number of subscribing societies is growing. The scope of Laboratory Animals is to publish all aspects of the use of animals in biomedical research, including: new animal models; laboratory animal microbiology; clinical case reports; descriptions of new or improved research techniques; reports on the influence of environmental and other variables on research results; descriptions of techniques which offer replacements for in vivo models; and basic data characterizing the haematology, biochemistry or pathology of new or existing animal models. Of special interest are papers that deal with the 3Rs (reduce, refine and replace) or leading to improvements in the welfare or well-being of the animals used. Laboratory Animals has well elaborated guidelines, and authors are strongly recommended to follow those guidelines as well the ARRIVE guidelines that help in the preparation of manuscripts on reporting animal data. Submitted
manuscripts are first screened for their suitability for the journal and then put through the peer review process. Over the past years more than 200 manuscripts have been submitted per year. Our goal is to give a first decision on manuscripts within 30 days. The acceptance rate is 34%. The goal of LAL is to make the journal attractive and to raise the impact factor by having reports on high-quality research. The current impact factor is 1.120, ranking the journal 71/153 in zoology and 53/133 in veterinary sciences. For a long time the publisher was the Royal Society of Medicine (RSM) Press Ltd, but several years ago SAGE Publications Press Ltd took over the journal. Next to scientific articles, the journal provides space for advertisements to promote the latest technology and commercial advertisements. For the journal this is important revenue which partially covers printing and distribution costs. Some of the profit is used to fund bursaries to promote projects that deal with continued education in animal welfare and to promote good laboratory animal science (see http://www.lal.org.uk/funding-and-opportunities/bursaries/).

Today, what counts is the visibility of the journal in the field of laboratory animal science, its impact factor, and the use of the profit for bursaries to promote animal welfare and the spirit of the 3Rs. It manages a fine balance between business and the promotion of animal welfare. For the 50th anniversary, Laboratory Animals will change its appearance, and will be published in six issues at bimonthly intervals. There will be several new features. In addition to reviews and working party reports, short reports, original work, letters and comments, abstracts will be translated into French, German and Spanish to make contributions accessible for more readers. For subscribing societies, there is a ‘News’ section for contributions (in English or other languages) that report on events and topics that may be of broad interest. As one may realize, this means extra work, and Laboratory Animals has increased its editorial board as well as editorial staff to maintain quality of publication and also to keep up with a tighter publication schedule. It is also planned for one of the six issues to invite guest editors to prepare an issue with mini reviews on selected topics.

Beat M Riederer
Editor-in-Chief, Laboratory Animals

Laboratory Animals Limited, the Company

Although a company, LAL is better known as a charity. Its charitable activities are its most important activity aside from the publication of Laboratory Animals (the journal). LAL is dedicated to the advancement of all aspects of laboratory animal science and welfare, and this is achieved through supporting education and training with the aim of improving scientific quality and animal welfare. LAL’s funding programme includes bursaries for individual scientists who wish to attend training (courses) in laboratory animal science and welfare. Bursaries are also provided to course organizers. LAL gives priority to those applications that meet the train-the-trainer concept. This way we hope to increase and maximize the action radius of our bursary programme, and hence spend our resources most effectively. Therefore we are also sympathetic to applications for funding the organization of events and conferences, albeit with conditions. Over the past 10 years LAL has given out over 600,000 euro in bursaries and for other activities promoting laboratory animal science and welfare, including attendance at and organization of courses, speakers, and audiovisual platforms. The 50th anniversary of LAJ is being celebrated with an increase in publication frequency of the journal from four to six issues a year. With a rising impact factor there is reason for optimism about the journal’s future. This is good news for the charitable activities of LAL. A few changes are being made to the priority areas of funding. Subscribing associations and their members will have priority. Areas, namely European countries, that have to build laboratory animal science education and training programmes from scratch as a result of the implementation of the European Directive 2010/63/EU will find a favorable ear with LAL’s funding programme. This includes the translation of teaching materials, guidelines and recommendations. LAL has decided to extend the yearly amount of money dedicated to the support programme if the financial situation allows us to do so. The success of the journal is, therefore, of pivotal importance. We wish LAJ a happy birthday and many more years of good works to come.

Jan-Bas Prins
Chairman of Laboratory Animals Limited
Gender differences in a mouse model of chemotherapy-induced neuropathic pain

H Naji-Esfahani¹,², G Vaseghi¹, L Safaeian³, A-A Pilehvarian², A Abed⁴ and M Rafieian-Kopaei⁵

Abstract
Chemotherapy-induced neuropathic pain is one of the major problems for cancer patients. Although paclitaxel and cisplatin are widely used in women, most laboratory studies of chemotherapy-induced neuropathic pain have been conducted on male animals. The current study examined the gender differences in chemotherapy-induced neuropathic pain in mice. Neuropathic pain was induced by intraperitoneal injection of paclitaxel (2 mg/kg) for five consecutive days and cisplatin (1 mg/kg) for seven consecutive days. Cold allodynia was evaluated by measuring the paw withdrawal frequency and duration of paw licking in mice; however, mechanical allodynia was assessed by von Frey filaments. Neuropathic pain began to manifest after a few days ($P < 0.001$). Cold allodynia was more robust in female mice ($P < 0.001$) treated with paclitaxel, while no differences were observed between the two genders in the manifestation of paclitaxel-induced mechanical allodynia. Interestingly, no gender differences were observed in cisplatin-induced cold and mechanical allodynia tests. In conclusion, gender differences play a major role in neuropathic pain induced by paclitaxel. The differences between male and female animals should be considered in future studies and the findings should be generalized to humans with caution.

Keywords
gender, chemotherapy, neuropathic pain

It is believed that gender differences exist in the pathology of chronic and neuropathic pain.¹ Various clinical studies have proposed that women are more sensitive to pain than men.²,³ Furthermore, animal studies have demonstrated that female rodents show a lower threshold for pain in different models; however, the underlying mechanisms are not well understood.⁴ Chemotherapy-induced neuropathic pain is one of the major side-effects in cancer treatment using taxane and platinum complexes.⁵ The pain is characterized by numbness, tingling, burning sensation and cold allodynia.⁶ Neuropathic pain may impair the patient’s quality of life and compromise a patient’s ability to tolerate chemotherapy.⁷ The risk of neuropathy is proportional to the dose and duration of administration of chemotherapeutic agents.⁸,⁹ There is no established approach to the prevention and treatment of chemotherapy-induced nerve damage.¹⁰

Paclitaxel and cisplatin cause significant neuropathic pain. These are major drugs for the treatment of breast and ovarian cancers and are widely used in female patients.¹⁰ Different animal models are available to investigate the effectiveness of these treatments.¹¹,¹² Rats and mice are frequently used to evaluate mechanical and cold allodynia tests.¹³ These tests are predominantly performed on male...
animals. Sometimes researchers ignore the role of gender in experiments whereas others believe that the female hormonal cycle can cause problematic variations in their findings. However, the relation between levels of chemotherapy-induced neuropathic pain and gender differences has not yet been studied in mice. In this study, we investigate the role of gender in paclitaxel- and cisplatin-induced neuropathic pain.

Materials and methods

Animals

NMRI mice (Pasteur institute, Tehran, Iran) weighing 20–30 g were used. The animals were housed under a 12 h light/dark cycle with free access to food and water, and were assigned to different treatment groups (n = 6–8 in each group). All experiments were conducted in accordance with the guidelines for the care of laboratory animals of the Ethics Committee of Isfahan University of Medical Sciences.

Drugs and drug administration

Cisplatin and paclitaxel were purchased from Sigma–Aldrich Inc (St Louis, MO, USA). Cisplatin was freshly dissolved in sterile saline, and paclitaxel was dissolved in ethanol–cremophor–saline (5:5:90, v/v/v). Male and female mice received paclitaxel (2 mg/kg) intraperitoneally for five consecutive days with a cumulative dose of 10 mg/kg, or vehicle. Cisplatin (1 mg/kg) was intraperitoneally administered to male and female mice for seven consecutive days. A control group received saline.

Behavioral assay

The mice were placed on top of an aluminum mesh table and allowed to adjust to the situation for approximately 15 min. Briefly, for the assessment of cold allodynia, acetone was applied three times via a needle and syringe to the plantar surface of each hind paw with 30 s intervals. The time spent licking the paw was recorded with a stopwatch. The frequency of licking was calculated and expressed as a percentage using the following formula (number of trial attending the hind paw/total number of trial) × 100. For assessment of mechanical allodynia, von Frey filaments (Stoelting, Wood Dale, IL, USA) ranging from 0.16 to 6 g were used as previously described. Bending force was applied to the plantar skin of the right hind paw, and each application was held for 6 s, using the up-down method to determine threshold sensitivity.

Data analysis

Data are presented as mean ± standard deviation, and were compared by one-way analysis of variance (ANOVA) followed by Fisher LSD post hoc test for multiple comparisons. Time-course analysis of behavioral data was compared by repeated measures ANOVA for each experimental group.

Results

Effects of paclitaxel on cold allodynia

Paw withdrawal frequency and duration of paw licking increased in both genders after treatment with paclitaxel (2 mg/kg, for 5 consecutive days). The control group did not show any changes in nociceptive response. Also paw withdrawal frequency and duration of paw licking were more robust in female mice compared with male mice or the control group (P < 0.001). Interestingly, pain was manifested sooner in the female mice than in the male mice (Figures 1a and 1b).

Effects of paclitaxel on mechanical allodynia

We evaluated withdrawal response to mechanical stimulation using von Frey filaments. Paclitaxel (2 mg/kg, for 5 consecutive days) led to a significant reduction in paw withdrawal threshold in male and female mice (P < 0.01) (Figure 2). However, we observed no significant differences in responses between male and female mice during this period of time.

Effects of cisplatin on cold allodynia

Administration of cisplatin (1 mg/kg) for seven days evoked cold allodynia in treatment groups (P < 0.001). Both paw withdrawal frequency and duration of paw licking increased in male and female mice. However, no significant differences were observed between the genders (Figures 3a and b).

Effects of cisplatin on mechanical allodynia

As shown in Figure 4, a statistically significant difference was observed in the manifestation of mechanical sensitivity between cisplatin-treated mice and the control group (P < 0.01), while no differences were observed between the genders (Figure 4).

Discussion

The aim of this study was to evaluate the effect of gender differences on levels of chemotherapy-induced neuropathic pain in mice. Paclitaxel and cisplatin are frequently used for the treatment of breast and ovarian
cancers; however, the vast majority of preclinical studies to identify the proper treatment for neuropathic pain have been conducted using male mice. In this study we sought to determine whether male mice are good candidates for these models of pain. We showed that paclitaxel-induced cold allodynia was more robust in female mice. Interestingly, no gender differences were observed for paclitaxel-induced mechanical allodynia and cisplatin-induced neuropathic pain.

Paclitaxel is used as a chemotherapeutic agent with different types of cancers such as breast, cervical and ovarian cancers. It enhances microtubule polymerization, but the mechanisms by which paclitaxel induces neuropathic pain have not yet been demonstrated. Paclitaxel induces degeneration of axonal microtubules but has no effects on the axonal cytoskeleton. We observed no differences in paclitaxel-induced mechanical allodynia between male and female mice; and this finding is consistent with a study which also showed no gender differences in paclitaxel-induced mechanical allodynia in rats. However, gender differences in cold allodynia were not consistent with previous studies, possibly suggesting different mechanisms are involved. The differences between mice and rats have already been noted during other behavioral studies. For example, periorbital and forepaw thresholds, but not hind paw thresholds, are reduced in mice with craniotomy in the migraine model. In contrast to mice, these effects are not observed in rats. It is believed that mouse species are more susceptible than rats to pain and stress. Behavioral differences also exist in response to stressors, and significant differences have
been noted among species and even strains within species.\textsuperscript{24} Cisplatin is widely used for the treatment of urinary, bladder, and ovarian cancers.\textsuperscript{25} It has been reported that sensory perception and sensory nerve conduction are reduced following treatment with cisplatin.\textsuperscript{26} Nerve biopsy evaluations have revealed loss of myelinated fibers.\textsuperscript{27} Analysis of dorsal root ganglion in animals has shown atrophy of neuronal soma and nucleus.\textsuperscript{28} Sexual dimorphism exists in peripheral nerve abnormalities. A study has demonstrated that mortality and weight loss are more robust in male mice treated with cisplatin.\textsuperscript{29} In the present study, we observed no differences between male and female mice in cisplatin-induced neuropathic pain. However, one other study has found faster regeneration and functional recovery in female animals.\textsuperscript{30} Vincristine induces neuropathic pain which is more severe in females than in males.\textsuperscript{31} suggesting that the impact of gender difference depends on the type of tests and models. A large body of evidence has suggested that endogenous sex steroid plays a major role in mediating sex differences in nociception. Pain modulation is more effective during the ovulatory phase than during the early follicular and mid-luteal phases.\textsuperscript{32} There are many possible mechanisms by which sex hormones may affect pain. For example, serotonergic and noradrenergic neurons in the nucleus raphe magnus and locus coeruleus express estrogen and progesterone receptors and interact with sex hormones, which leads to the release of opioids in the spinal cord.\textsuperscript{33} Interaction with N-methyl-D-aspartate (NMDA) receptors in the spinal cord/brainstem is another mechanism; NMDA receptor antagonists reduce the activity of neurons in trigeminal subnucleus caudalis in an estrogen-dependent manner.\textsuperscript{34} Experimental hyperalgesia models have shown significant gender differences; however, not all pain animal

![Figure 3](image-url) (a) Paw withdrawal threshold frequency after cisplatin treatment. Cisplatin [1 mg/kg] was injected for seven consecutive days, and the control group received vehicle. No significant gender difference was observed for cisplatin-induced cold allodynia ($^\times P < 0.01$, $^\times \times P < 0.001$ when compared with the control group). (b) Duration of paw licking after cisplatin treatment. Cisplatin [1 mg/kg] was injected for seven consecutive days, and the control group received vehicle. No significant gender difference was observed for cisplatin-induced cold allodynia ($^\times \times P < 0.001$ when compared with the control group).

![Figure 4](image-url) Paw withdrawal threshold frequency after cisplatin treatment. Cisplatin [1 mg/kg] was injected for seven consecutive days, and the control group received vehicle. No significant gender difference was observed for cisplatin-induced mechanical allodynia ($^\times P < 0.01$ when compared with the control group).
models show robust gender differences. Clinically, pain is more pronounced in women, but few pain conditions are predominant in men, including post traumatic headaches and abdominal migraines. These studies suggest that different mechanisms and risk factors may be involved in each gender.

In conclusion, our study demonstrated that there was no significant gender difference in a mouse model of cisplatin-induced neuropathic pain; however, a gender difference was observed in paclitaxel-induced cold, but not mechanical, allodynia.

It may therefore not be advisable to evaluate different analgesics in male animals, as there are many differences between genders and among different models. Human studies should also be performed and the results should be compared with animal studies to determine the best model of pain for evaluating different drugs.

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Declaration of conflicting interest
None.

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References


Effects of type of light on mouse circadian behaviour and stress levels

Marta Alves-Simões, Georgia Coleman and Maria Mercè Canal

Abstract
Light is the principal synchronizing environmental factor for the biological clock. Light quantity (intensity), and light quality (type of light source) can have different effects. The aim of this study was to determine the effects of the type of light experienced from the time of birth on mouse growth, circadian behaviour and stress levels. We raised pigmented and albino mice under 24 h light–dark cycles of either fluorescent or white light-emitting diode (LED) light source during the suckling stage, and the animals were then exposed to various light environments after weaning and their growth rate, locomotor activity and plasma corticosterone concentration were measured. We found that the type of light the animals were exposed to did not affect the animals’ growth rates or stress levels. However, we observed significant effects on the expression of the locomotor activity rhythm under low contrast light–dark cycles in pigmented mice, and under constant light in both albino and pigmented mice. These results highlight the importance of environmental light quality (light source) on circadian behavioural rhythms, and the need for close monitoring of light environments in animal facilities.

Keywords
mouse, albino, pigmented, fluorescent light, LED light

Mammals have developed an autonomous endogenous clock in their brain to coordinate and anticipate their physiology and behaviour to the daily circadian changes in light and darkness in the environment, induced by the earth’s rotation. The principal circadian pacemaker is located in the brain suprachiasmatic nucleus (SCN).1 Light is the most important synchronizing environmental factor for the circadian clock. Photic information is detected by the photoreceptors in the retina and is then transmitted, via the retinohypothalamic tract, to the SCN. The SCN will use this information to synchronize (entrain) the animal’s circadian rhythms to the environment. Thus light has the ability to influence physiology and behaviour in animals including humans.

In addition to the classical photoreceptor rods and cones, which collect and process light to generate an image of the world, there is a third type of photoreceptor, the intrinsically photosensitive retinal ganglion cells (ipRGCs), which are key to measuring environmental irradiance, and which then modulate physiological responses to light including photoentrainment.5 Studies in transgenic rd/rd e1 mice, whose retinas lack rods and cones, have shown that these animals are able to regulate their circadian rhythms with the same sensitivity as fully sighted animals,3 and have thus demonstrated that ipRGCs play an important role in the regulation of the circadian system and photoentrainment. ipRGCs are particularly sensitive to blue light, as they contain the photopigment melanopsin, which has a peak sensitivity of almost 480 nm.4 Therefore, light photoreception is not only sensitive to irradiance levels (melanopsin phototransduction is only engaged at moderate to high irradiance3), but also has spectral sensitivity, defined by the spectral efficiency of the photopigments expressed within rods, cones and ipRGCs.6 This means that environmental light with higher irradiance and shorter visible wavelength range will have a more pronounced effect on...
ipRGCs and consequently, on the circadian system. Therefore, light source will play an important role in experimental animals exposed to artificial lighting (e.g. fluorescent or light-emitting diode [LED]), which has a spectral distribution different to that of natural sunlight.

The circadian system of mammals is immature at birth and they finish their maturation postnatally. In the womb and at birth, the pup's circadian rhythms are synchronized to the environment through various maternal signals. In rats and mice, clock gene rhythms start to be affected by the photoperiod at postnatal day 10, coinciding with the time when most connections to and from the SCN, including the retinohypothalamic tract, have reached adult patterns with eyes open, and thus the significance of maternal influence starts decreasing in parallel with an increasing effect of external photic stimuli by around the second postnatal week. Interestingly, there is a difference in the rate of development of the various photoreceptors: the development of ipRGCs in rodents starts from around the time of birth, long before rods and cones develop, which suggests that a particular environmental light type which targets ipRGCs may exert more pronounced effects during development than a light type which targets rods and cones.

The aim of this study was to determine the effects of light type experienced from birth on circadian rhythm function. We used both albino and pigmented mice, as albino mice are more sensitive to environmental light damage and may thus have different sensitivities to light type compared with pigmented mice. Postnatal light environment can affect growth rates and stress levels in rodents, and exposure of adult mice to altered light–dark cycles or constant light increases corticosterone concentrations, and anxiogenic and depressive-like behaviours. Therefore, we also tested the effects of postnatal light types on growth rates, and plasma corticosterone concentrations in mice kept in different light environments (light–dark cycles, constant dark and constant light).

**Animals, materials and methods**

**Animals and experimental design**

Four pregnant female mice (two C57BL/6J and two CD1) raised in our colony were kept in a fluorescent light environment, and five additional pregnant female mice (three C57BL/6J and two CD1) in a LED light environment under 12:12 h light–dark cycles (LD, L: 28 \mu W/cm^2, D: 0 \mu W/cm^2) until the day of delivery (see details on light sources below). During the suckling stage – from the day of birth (postnatal day 0, P0) until weaning on P23 – pups were kept with their dams in their respective light environments.

On the day of weaning (P23), pups were moved to individual cages. A total of 28 female pups were used: six LED-raised C57BL/6J mice, nine LED-raised CD1 mice, four fluorescent-raised C57BL/6J mice and nine fluorescent-raised CD1 mice. Mice were exposed to four successive experimental stages (Figure 1): stage 1 (LDm, P23–P37, LD cycles of medium-light intensity [28 \mu W/cm^2]), stage 2 (DD, P38–P53, constant darkness), stage 3 (LDl, P54–P92, LD cycles of low-light intensity [2.8 \mu W/cm^2]), and stage 4 (LL, P93–P114, constant light of low-light intensity [2.8 \mu W/cm^2]). During stages 1 to 4, all the mice were kept in the same light type (fluorescent or LED) that they had been raised in during the suckling stage.

Locomotor activity was recorded continually throughout the experiment by means of infrared activity meters placed outside the animals’ cages. Data were stored in a computer in 15 min bins, and analysed thereafter. At the end of each experimental stage, a blood sample was taken from each mouse to analyse its corticosterone plasma concentration. Its body weight was measured once a week from weaning until the end of the experiment. Throughout the experiment, the mice

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**Figure 1.** Experimental design. The animals were raised under 24 h LD cycles of either fluorescent or white light-emitting diode [LED] light during the suckling stage. They were then exposed to various light environments from weaning, of the same light type than that experienced during the suckling stage. LD: 24 h light–dark cycle (12:12 h); DD: constant dark; LL: constant light; l: low-light intensity [2.8 \mu W/cm^2]; m: medium-light intensity [28 \mu W/cm^2]; P: postnatal day.
were maintained under a controlled ambient temperature of 21 ± 2°C and relative humidity of 60 ± 5%, with water and food (B&K Universal, Hull, UK) ad libitum. At the end of the experiment, the mice were culled by cervical dislocation. All experimental procedures were conducted in accordance with the United Kingdom Animals (Scientific Procedures) Act 1986.

**Light sources**

Throughout the experiment, the animals were kept in light-tight ventilated cabinets (Tecniplast, Buguggiate, Italy) with either a white fluorescent (FH14W/840 HE; Osram, Milan, Italy) or cold white LED (OSM1P/30; VLM, Hilden, Germany) light source. Although the light intensity changed during the experiment (see above), it was always the same in both the fluorescent and LED cabinets at each stage of the experiment.

Light spectral distribution in each cabinet was measured before the start of the experiment by means of a spectrometer (Ocean Optics, Dunedin, FL, USA) to confirm the differential spectral distribution between the fluorescent and LED light sources (Figure 2).

**Behavioural analysis**

The chronobiology software package El Temps (A Diez-Noguera, Universitat de Barcelona, Barcelona, Spain) was used to calculate several variables of the motor activity rhythm. The period of the motor activity rhythm was calculated using the \(X^2\)-periodogram. The percentage of variance (PV) explained by the highest peak obtained in the periodogram was used as an indicator of the stability of the circadian rhythm of locomotor activity. We used the mean wave form of daily activity to study the characteristics of the alpha or active phase. Alpha was defined as the period of time during the daily cycle in which most of the activity levels were above the median. The duration of the alpha phase in relation to the duration of the daily cycle (%) was compared between groups. The activity levels during the active phase in relation to the total daily activity levels (%) were also calculated.

**Corticosterone analysis**

At the end of each experimental stage, a blood sample from the tail vein was taken from each mouse to analyse its plasma corticosterone concentration. To account for potential influences of the oestrus phase on corticosterone levels, blood sampling was performed at the trough of the corticosterone circadian rhythm (circadian time 2 [CT2] or zeitgeber time 2 [ZT2], which corresponds to 10 h before the onset of activity at CT/ZT12), as the plasma corticosterone concentration at this time point is similar throughout the various phases of the oestrous cycle in females, and similar to that in males.\(^{17}\) Blood samples were mixed with 3 μL of 5 mM EDTA (Sigma-Aldrich, Gillingham, UK), spun at 2656 g for 15 min, and frozen (−80°C) until used. An enzyme immunoassay (Cayman Chemical Company, Ann Arbor, MI, USA) was used in accordance with the manufacturer’s instructions to determine corticosterone concentration. The 96-well plates were read with the ‘Gen5’ software package (BioTEK Instruments Inc, Winooski, VT, USA) at 405 nm (plate reader Synergy HT; BioTEK).

**Data and statistical analysis**

For all experimental stages, ‘light type’ refers to the type of light source (fluorescent versus LED) in which the mice were raised in during the suckling period. This light type corresponds with the same light type the animals were subsequently exposed to in the experimental stages LDm, LDl and LL.

SYSTAT version 10 (SPSS Inc, Chicago, IL, USA) was used to analyse all data. A general linear model was used to analyse locomotor activity rhythms and corticosterone concentrations. The independent variables were the light type during the suckling stage (fluorescent or LED) and the experimental stage (LDm, DD, LDl, LL); interactions between the independent variables were also included in the model. Two models were run, one for each mouse strain (CD1 and C57BL/6J). An analysis of variance (ANOVA) of repeated measures was used to test the effect of postnatal light type on growth rate. When a statistically significant difference was encountered, a subsequent Bonferroni post hoc test was applied. Significance was set at \(P < 0.05\) for all statistical tests.

![Figure 2](image_url). Spectral distribution of the fluorescent and light-emitting diode (LED) light sources used in this experiment.
Results

In this experiment, pups were either raised under fluorescent or LED light–dark environments throughout the suckling period, and then kept under various light environments of the same light type until the end of the experiment (see Figure 1 for experimental design). Figure 2 shows the spectral distribution of the fluorescent and LED light sources used.

Type of light effects on body weight

Body weight was examined weekly from weaning until the end of the experiment. We found that, both in C57BL/6 J and CD1 mice, the types of light the animals were kept in had no effect on their body weights or growth rates (Figure 3).

Type of light effects on behaviour

Locomotor activity was recorded constantly throughout the experiment (Figure 4). Four key aspects of the circadian rhythm of locomotor activity were examined: period, strength (as indicated by the PV of the highest peak in the periodogram), and duration and amount of locomotor activity performed during the active (alpha) phase of the rhythm.

In C57BL/6 J mice, the experimental stage had a statistically significant effect on the period (\( P < 0.001 \), Figure 5a) and PV (\( P < 0.001 \), Figure 5c) of the locomotor activity rhythm, and also on activity duration (\( P = 0.010 \), Figure 5e), but not on activity levels (Figure 5g). We found by post hoc analysis that the period in the LL stage was longer than all the other stages (\( P < 0.001 \)). The strength of the rhythm or PV was higher in the LDl stage compared with all the other stages (\( P < 0.01 \) versus DD, LL; \( P < 0.05 \) versus LDm). The duration of the active phase was significantly shortened in the LL stage compared with the LDl and DD stages (\( P < 0.05 \)).

Regarding the effects of type of light, we found a significant interaction between the experimental stage and the light type, with mice kept in LED light showing longer periods than mice kept in fluorescent light, but only in the LL stage (\( P < 0.001 \), Figure 5a). We also found that, in the LDl stage, mice kept under LED light had stronger rhythms (higher PV values) than those kept under fluorescent light (\( P < 0.05 \), Figure 5e).

In CD1 mice, we found a significant effect of the experimental stage on period (\( P < 0.001 \), Figure 5b). Post hoc analysis showed that the free-running period in the LL stage was significantly longer than that of all the other stages (\( P < 0.001 \)). PV also depended on the experimental stage (\( P < 0.001 \), Figure 5d). Specifically, we found that PV was lower in the LL stage compared with all the other stages (\( P < 0.001 \)). The duration of the active phase was also shorter in the LL stage than in all the other experimental stages (\( P < 0.001 \), Figure 5f), and also shorter in the DD stage compared with the LDm stage (\( P < 0.05 \)). The amount of activity performed during the alpha phase also depended on the experimental stage (\( P < 0.001 \), Figure 5h), with lower activity levels in the LL stage compared with all the other stages (\( P < 0.001 \)). In addition, we found that the type of light the animals were kept in had statistically significant effects on the period of the locomotor activity rhythm in the LL stage, so that the free-running period was longer in the mice kept in

Figure 3. Growth rates. Body weight increase throughout the experiment of C57BL/6 J (a) and CD1 (b) mice kept in either fluorescent or white light-emitting diode (LED) light from birth.
Figure 4. Locomotor activity rhythm. Representative actograms of C57BL/6 J (a, c) and CD1 (b, d) mice kept in either fluorescent (a, b) or white light-emitting diode (LED) (c, d) light from birth. The actograms show the animals’ locomotor activity rhythm throughout the various experimental stages. LD: 24 h light–dark cycle (12:12 h); DD: constant dark; LL: constant light; l: low-light intensity (2.8 μW/cm²); m: medium-light intensity (28 μW/cm²).
Figure 5. Locomotor activity rhythm analysis. Main circadian rhythm characteristics in each experimental stage in C57BL/6 J and CD1 mice raised under a fluorescent or light-emitting diode (LED) light during the suckling stage. (a, b) Period. (c, d) Percentage of variance [PV]. (e, f) Duration of daily active phase. (g, h) Activity levels during the active phase. Data presented as mean ± standard error \( n = 4-10 \). * \( P < 0.05 \), ** \( P < 0.01 \), *** \( P < 0.001 \) Fluorescent versus LED; * \( P < 0.05 \), ** \( P < 0.01 \), *** \( P < 0.001 \). LD: 24 h light–dark cycle (12:12 h); DD: constant dark; LL: constant light; l: low-light intensity \( 2.8 \mu \text{W/cm}^2 \); m: medium-light intensity \( 28 \mu \text{W/cm}^2 \).
LED light than in those kept in fluorescent light ($P < 0.01$, Figure 5b).

**Type of light effects on stress levels**

We determined stress levels by examining corticosterone plasma concentrations at the end of each experimental stage. In the C57BL/6 J mice, we found that corticosterone plasma concentration was independent of the experimental stage and type of light experienced (Figure 6a). In the CD1 mice, the experimental stage had a statistically significant effect on the plasma corticosterone concentration ($P < 0.01$, Figure 6b), however significance was lost when running the post hoc test, which suggests that the difference between stages is weak. The type of light the mice were kept in had no significant effect on plasma corticosterone concentration (Figure 6b).

**Discussion**

We investigated the effect of light type experienced from birth on circadian behaviour, growth rates and stress levels in albino and pigmented mice. We found no effects of postnatal light type on growth rates or stress levels under different light environments; however we found significant effects on circadian behaviour in both CD1 and C57BL/6 J mice.

We found no effects of light type experienced during postnatal development on body weight after weaning. This is in contrast to Borniger et al., who found that female mice raised under LD cycles with dim light at night had significantly lower body mass than control mice exposed to LD cycles with darkness at night after weaning (postnatal weeks 5 and 7), but no differences were observed later on. The main difference between the two experiments is that while our animals were exposed to LD cycles with light during the day and darkness during the night, the mice in the study by Borniger et al. were not. These results suggest that continuous illumination during postnatal development may slow down the initial growth rate in newborn pups, but alternating LD cycles of either fluorescent or LED light and darkness do not.

Studies raising rodent litters under extreme light conditions during the postnatal period (i.e. LL, DD) have shown that postnatal light environments (and not maternal circadian rhythms) have a long-term effect on the future expression of the pup’s circadian rhythm. Here, all the litters were exposed to normal LD cycles with medium-light intensity during pregnancy and the suckling stage, and thus they were all synchronized to a 24 h rhythm, suggesting that no major differences in maternal circadian rhythms occurred between the groups.

As expected, exposure of CD1 mice to LL significantly lengthened their free-running period (tau) and weakened their circadian rhythms of locomotor activity. We also found LL induced negative masking in albino mice, as indicated by the significant reduction in activity duration and activity levels in the LL stage compared with the other experimental stages. Interestingly, we found that the type of light experienced during the suckling stage particularly targeted the locomotor activity rhythm in LL. Indeed, in the LL stage, the tau was longer in LED-raised compared
with fluorescent-raised mice. According to Aschoff’s rule, tau in LL lengthens with increased light intensity, and since all the animals were exposed to the same light intensity, the results suggest that CD1 LED-raised mice perceived a higher light intensity than CD1 fluorescent-raised mice. This could be explained by the fact that white LED light contains more blue light than fluorescent light (Figure 2), and since ipRGCs sensitivity peaks around 480 nm (blue light), the signal received by ipRGCs and transmitted to the SCN may be stronger under LED light compared with fluorescent light.

In C57BL/6 J mice, we also found a tau lengthening when the animals were exposed to LL. Similarly to CD1 mice, the tau of the locomotor activity rhythm of the mice exposed to LED light in the LL stage was longer than that of the mice kept in fluorescent light. In addition, we found that the LED-raised mice showed stronger rhythms than the fluorescent-raised mice in the LDl stage. These results suggest: (a) mice kept in LED light may perceive higher light intensities, and (b) a better ability of pigmented LED-raised mice to entrain to low contrast LD cycles. Again, these results could be due to the fact that white LED light has a higher proportion of blue light in its composition than fluorescent light (Figure 2), thus giving a stronger signal initially to the ipRGCs, and ultimately the SCN.

The tau length of an animal exposed to constant darkness is species-specific, and thus it is not surprising that the period in DD differs between C57BL/6 J and CD1 mice. Inter-strain differences in the tau in DD have been associated with differences in responses to environmental light, which could explain the slight differences found in the expression of the circadian rhythm of locomotor activity in LL and LDl between C57BL/6 J and CD1 mice. Nevertheless, no effects were found in either mouse strain resulting from the light type the animal was exposed to from birth on the tau in DD, which suggests that light type does not affect the intrinsic function of the circadian pacemaker.

Changes in the amount of light a rodent is exposed to during postnatal development (the photoperiod) have been shown to have long-term effects on plasma corticosterone concentrations and anxiety-like behaviour later in life. In this study we examined the effects of light type on stress levels by determining plasma corticosterone concentrations at the end of each experimental stage. The entire procedure of blood collection, from opening the cabinet housing the mouse cages to putting the blood on ice took 3–4 min, thereby avoiding stress-induced increases in corticosterone. This is demonstrated by the finding that corticosterone measurements at ZT/CT2 were between 10–40 ng/mL in this study, which is in line with the published literature, and significantly lower than a stress-induced plasma corticosterone concentration (>1000 ng/mL). We found no significant changes in overall corticosterone plasma concentrations due to light type in both C57BL/6 J and CD1 mice. In addition, we found no significant differences in plasma corticosterone concentrations due to the experimental stage. This is interesting, as constant light exposure has been shown to increase overall plasma corticosterone concentrations. However this study was performed in rats. In mice, six weeks of LL exposure has been shown to actually decrease plasma corticosterone concentrations, possibly due to the down-regulation of the hypothalamic–pituitary–adrenal axis (HPA). Thus it is possible that two weeks of LL exposure in our study was not a long enough period of time to induce depression of the HPA axis. Taken together, these results suggest no major changes in the stress responses due to various light environments and light sources in both mouse strains in our experimental conditions.

Due to lack of pigment, albino retinas are more sensitive than pigmented retinas to damage by environmental light received either during postnatal development or in adulthood. We thus hypothesized that albino CD1 mice would be more sensitive than C57BL/6 J mice to the effects of light type in this experiment. The finding that the growth rates and stress levels in both mouse strains were unaffected by light type or experimental stage, and that the free-running period of the locomotor activity rhythm in the LL stage was longer in the LED-kept mice of both strains, suggest similar effects of light type on albino and pigmented mice. However, the finding that pigmented mice kept under LED light have stronger rhythms than those kept in fluorescent light in a low-contrast light–dark cycle (LDl stage), suggests that pigmented mouse retinas may in fact be more sensitive to spectral changes in light input.

Both the amount of light and the timing of the light exposure received during the suckling period have been previously shown to have long-term consequences on the expression of circadian behavioural rhythms in rodents. In this study we have found that circadian behavioural rhythms of both albino and pigmented mice are affected by the light type they are exposed to from birth. The circadian system appears to be particularly sensitive to light type under low contrast LD cycles and under constant light. Additional experiments are required to further elucidate the role of type of light on the programming of the developing circadian system. Nevertheless, we did not find that the type of light had any significant effects on growth rates or stress levels in the LD and DD stages, or in response to LL exposure. These results highlight the importance of environmental light quality and quantity on circadian
behavioural rhythms, and the need for close monitoring of light environments in animal facilities.

**Conflict of interest**
None declared.

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**References**
Assessing the welfare of laboratory mice in their home environment using animal-based measures – a benchmarking tool

Elin MF Spangenberg and Linda J Keeling

Abstract
Welfare problems in laboratory mice can be a consequence of an ongoing experiment, or a characteristic of a particular genetic line, but in some cases, such as breeding animals, they are most likely to be a result of the design and management of the home cage. Assessment of the home cage environment is commonly performed using resource-based measures, like access to nesting material. However, animal-based measures (related to the health status and behaviour of the animals) can be used to assess the current welfare of animals regardless of the inputs applied (i.e. the resources or management). The aim of this study was to design a protocol for assessing the welfare of laboratory mice using only animal-based measures. The protocol, to be used as a benchmarking tool, assesses mouse welfare in the home cage and does not contain parameters related to experimental situations. It is based on parameters corresponding to the 12 welfare criteria established by the Welfare Quality® project. Selection of animal-based measures was performed by scanning existing published, web-based and informal protocols, and by choosing parameters that matched these criteria, were feasible in practice and, if possible, were already validated indicators of mouse welfare. The parameters should identify possible animal welfare problems and enable assessment directly in an animal room during cage cleaning procedures, without the need for extra equipment. Thermal comfort behaviours and positive emotional states are areas where more research is needed to find valid, reliable and feasible animal-based measures.

Keywords
laboratory animal welfare, welfare assessment, housing, husbandry, handling techniques, refinement

The welfare of laboratory rodents has long been a focus of attention. Refinement work within the 3R (replacement, refinement and reduction) concept is used ‘to improve scientific procedures and husbandry which minimize actual or potential pain, suffering, distress or lasting harm and/or to improve animal welfare in situations where the use of animals is unavoidable’. Many of the indicators of poor welfare identified for laboratory mice, such as stereotypic behaviours, barbering of whiskers or fur, and aggression between group-housed males, occur irrespective of the type of research. They occur in the home cage where the animal spends the majority of its lifetime, and ‘refinement’ applies to the lifetime experience of the animal. Furthermore, factors such as the progression of disease within an animal model or the effects of experimental procedures may themselves lead to deteriorated welfare in the home cage. If so, the cage environment needs to be adapted to meet the specific needs of these animals (e.g. easier access to food and water, softer bedding) in order to reduce the risk of poor welfare. Assessment protocols that aim to give an overview of the welfare Department of Animal Environment and Health, Swedish University of Agricultural Sciences, Uppsala, Sweden

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of laboratory rodents have been presented in recent years, but to our knowledge, with one exception, they are related to experimental procedures or specific animal models, rather than to a general assessment of the welfare in the home cage. There is as yet no generally accepted protocol that can be used for benchmarking welfare outcomes in an animal facility. This paper is part of a larger project and the protocol presented here has been tested in practice at three animal facilities in Sweden.

Appropriate resources and management, such as suitable bedding, nesting material and husbandry routines are preventive measures, or inputs, which reduce the risk of poor animal welfare. However, no matter how good or well thought out this input is, animals of different strains, sexes and ages will react differently to the input, based on genetic background and prior experiences, leading to different welfare outcomes for different individuals. In addition, even apparently similar environments may be managed differently by animal caretakers, further affecting the animals’ experience of a particular situation. This means that when assessing animal welfare it is important to measure the outcomes on the animals themselves, using animal-based measures. Animal-based measures can be applied regardless of housing and management routines and are sensitive to variations in management and to environment–animal interactions. A way of identifying the strengths and weaknesses of husbandry systems in terms of how they impinge on the animals is by using animal-based measures. Therefore, the protocol presented here uses only animal-based measures to assess animal welfare.

When developing this protocol, the intention was to enable identification of possible welfare problems and to benchmark these as animal welfare outcomes for comparisons over time. By assessing welfare at regular intervals, this protocol can be used as an ongoing management tool to document changes in the welfare of animals at a facility. Benchmarking is a way of evaluating current performance, relative to others or oneself, and of identifying areas for improvement. The protocol could be used in all types of laboratory rodent facilities and so be part of the official inspection of the facilities by the regulator or competent authority, even if it may not be necessary to check all measures in order to comply with legislation. The protocol could also be adapted for use in the assessment of the actual severity of experimental procedures. It is further proposed that the work behind establishing this protocol could even be useful for targeting future research areas by leading to the identification of areas where more research is needed to find valid (and feasible) animal-based measures of assessing the welfare of laboratory mice.

The idea behind the protocol

The protocol is based on the concept developed in the Welfare Quality® project. This concept consists of four welfare principles, namely good housing, good feeding, good health, and appropriate behaviour. The principles are divided into 12 welfare criteria to cover all aspects of the principles and hence give a full picture of the welfare status of the animals (Table 1). It is also inherent in the Welfare Quality® approach that the criteria should be assessed with animal-based measures whenever possible and that scores of good welfare in some criteria should not compensate for scores of poor welfare in other criteria.

The protocols that are already available for assessing laboratory animal welfare have not selected their measures based on these 12 welfare criteria, and so it is not clear whether they cover all the different areas of animal welfare. Hawkins et al. mentioned the concept of five domains of potential welfare compromise. These domains resemble the four principles of the Welfare Quality® project; however Hawkins et al. did not propose parameters related to these domains. Some protocols are designed specifically for mice, and some for specific disease models, while another protocol gives more general principles for effective welfare assessment of laboratory animals of all species. There are also protocols available for monitoring the development of pups from birth to weaning, which is especially relevant in the production of new genetically-modified lines of mice to identify possible deviations from normal development. In addition, several ‘informal’ protocols exist, such as routines for post-operative care, which can be specific for a certain animal model.

Paster et al. used animal-based measures to monitor mice in an abdominal tumour model and define humane endpoints. The protocol by Hawkins et al. emphasized the importance of relevant animal welfare measures (for any species), and proposed that a staff team approach (where different competences are of value) gives the most effective assessment. Evidently, animal-based measures are already being used in laboratory animal science, in the day-to-day assessment of welfare outcomes as a result of an experimental input. But when it comes to the home cage environment and the daily life of the animals outside the experimental situation, it is common to relapse into using resource-based and management-based measures such as cage changing intervals, types of bedding, access to nesting material or nest boxes, and group size when assessing welfare. As an example, in the accreditation of animal facilities, the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International) uses mainly parameters related to resources and management when evaluating the cage environment. But the choice of resources and husbandry routines is often governed by traditions and a combination of
scientific and practical considerations. Although these choices may be knowledge-based, they are not systematically assessed for animal-based outcomes (even though animal caretakers perform cage-side animal-based observations as a matter of course), and are not systematically benchmarked over time. Of the published welfare assessment protocols for laboratory animals, the one by Leach et al. is the one most similar to the protocol presented in this paper. These similarities are:

1. It is an assessment protocol that is intended to be used for benchmarking, and for comparisons between facilities and within facilities.

2. It assesses the welfare effects on the mice of housing and husbandry routines, and not of experimental situations.

3. It uses animal-based measures.

Our protocol is different to the one presented by Leach et al. in that:

1. Our protocol contains only animal-based measures, while the one by Leach et al. uses many resource and management-based parameters (e.g. resources available in cages, such as nesting material or shelters, and the staff’s interaction with the mice).

### Table 1. Overview of welfare principles, criteria and corresponding parameters chosen for assessing welfare in mice.

<table>
<thead>
<tr>
<th>Welfare principles</th>
<th>Welfare criteria</th>
<th>Mouse parameters</th>
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<tbody>
<tr>
<td>Good feeding</td>
<td>Absence of prolonged hunger</td>
<td>Body condition</td>
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<td></td>
<td>Ability to reach the food hopper (CCP at weaning)</td>
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<tr>
<td>Good housing</td>
<td>Absence of prolonged thirst</td>
<td>Dehydration</td>
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<td></td>
<td>Ability to reach the water nipple (CCP at weaning)</td>
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<td>Good health</td>
<td>Comfort around resting</td>
<td>Nest building performance</td>
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<td></td>
<td>Thermal comfort</td>
<td>Pups outside the nest</td>
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<td></td>
<td>Ease of movement</td>
<td>Gait/movements</td>
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<td></td>
<td>Absence of injuries</td>
<td>Lameness</td>
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<td></td>
<td>Piloerection</td>
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<td></td>
<td>Hunched position</td>
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<td></td>
<td>Wounds (excluding bite wounds)</td>
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<tr>
<td>Appropriate behaviour</td>
<td>Absence of diseases</td>
<td>Urine and faeces</td>
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<td></td>
<td>Coat condition</td>
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<td>Ocular/nasal discharge</td>
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<td></td>
<td>Distended abdomen</td>
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<td>Other deviations (innate/acquired)</td>
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<tr>
<td></td>
<td>Absence of pain induced by management procedures</td>
<td>Activity and interaction with environment</td>
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<td></td>
<td>Facial expressions of pain</td>
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<td></td>
<td>Expression of social behaviours</td>
<td>Whisker and/or fur trimming</td>
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<td>Bite wounds/marks</td>
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<td>Vocalizations/audible fights in cage</td>
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<td>Blood stains in cage</td>
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<td></td>
<td>Expression of other behaviours</td>
<td>Circling</td>
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<td>Jumping against cage wall</td>
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<td></td>
<td>Bar chewing</td>
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<tr>
<td>Good human–animal relationship</td>
<td>Good human–animal relationship</td>
<td>Approaching hand in cage</td>
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<td>Ease of handling when moving mice from dirty to clean cage</td>
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<td></td>
<td>Urination/defecation during handling</td>
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<td>Positive emotional states</td>
<td>Positive emotional states</td>
<td>Rearing</td>
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</table>
2. Our protocol is tested for inter-observer reliability (using Fleiss’ kappa and Kendall’s coefficient of concordance),\(^8\) while the one by Leach et al. is not.

3. The protocol by Leach et al. only assesses welfare at the group level (animal unit) while our protocol assesses both the group and individual animals.

However, the major difference between the two protocols is in the procedures for choosing the parameters. Leach et al.\(^11\) chose parameters through a consultation process with experts who were presented with parameters collected through a literature search. The parameters in our protocol were chosen on the basis of the 12 welfare criteria from the Welfare Quality\(^\circledast\) project to cover the different dimensions of welfare.

Materials and methods

Selection process for parameters

The parameters selected for each of the 12 welfare criteria are presented in Table 1. The first step of the selection process was to review existing protocols (published, web-based and informal) for animal-based measures. Thereafter these parameters were sorted according to the welfare criteria to identify which criteria might lack existing parameters. In total, 287 unique animal-based measures were listed, of which 224 (78%) were related to disease and injuries (which are two of the 12 welfare criteria). The remaining 63 parameters represented the remaining 10 criteria. For the criteria ‘absence of prolonged thirst’, ‘thermal comfort’, and ‘ease of movement’ only one animal-based measure per criterion was found in existing protocols; ‘saggy skin’ (dehydration),\(^22\) ‘pups outside nest’\(^17\) and ‘normal gait/movements’ (unpublished clinical score sheet), respectively. For the criteria ‘comfort around resting’ and ‘good human-animal relationship’ only two animal-based measures per criterion were found in the existing protocols; ‘differences in resting posture’\(^22\) and ‘use of nesting material’\(^11\) plus ‘response to capture handling’\(^11\) and ‘response to observer’,\(^11\) respectively.

If there was more than one animal-based measure, the next step was to choose which parameter(s) to use for each criterion. The protocol is intended to be used directly in an animal room in connection with cage cleaning procedures and without any need for extra equipment, which meant that several parameters had to be discarded already at this stage. For example, Hawkins et al.\(^5\) suggested animal-based measures of welfare such as heart rate, blood pressure and level of stress hormones be used if these were available as part of the scientific procedure. However, physiological parameters such as these are not suitable for the protocol in the current paper for the reasons stated above. This exclusion of parameters led to another literature search for alternative parameters for those criteria for which there was no longer a parameter and also for ‘new’ parameters that might have been published after the existing protocols had been designed. In a final step, parameters were selected on the basis of their reliability and whether or not they were feasible. Those with high documented reliability from previous studies were more likely to be chosen.

Several parameters, especially those related to injury, disease, or pain, could have been allocated to several of the welfare criteria. In the Welfare Quality\(^\circledast\) protocols each parameter can only be used to represent one criterion,\(^23\) since the scores are to be integrated to an overall welfare classification for a farm. Double criteria representation would mean double counting of that score. In this protocol, no overall welfare score is envisaged; however the specificity of the measures is still important in order to identify areas of better or poorer welfare. Also in the future other researchers might be interested in calculating an overall welfare score. For the parameters in question (e.g. ‘piloerection’ and ‘hunched position’ are commonly used signs of severe suffering, no matter what the cause), decisions therefore had to be made as to which criteria they should represent. The parameters needed to be able to identify any problems which might exist. Thereafter further testing, and possibly extra equipment, might be needed to find the reasons behind the problems.

Additional methodological points

Two parameters in the table are listed as ‘critical control points’ (CCP), namely ‘ability to reach the food hopper’ and ability to ‘reach the water nipple’. This concept originates from ‘hazard analysis and CCP’ (HACCP). This is a tool for assessing hazards and for establishing control systems that focus on prevention rather than relying mainly on end-product testing, e.g. for food safety. It is defined as ‘a point, step or procedure at which controls can be applied and is essential for preventing or eliminating a (food) safety hazard or reducing it to an acceptable (critical) level’\(^24\). For pups, the parameters in question should be tested at a specific time point, namely weaning, when pups must rely on their own capacity to find (reach) food and water. If this fails, the feeding and watering regimes need to be rejected and other methods have to be used. In this case it could mean that food should be placed on the cage floor and an extra-long water nipple should be installed.

In addition to the listed parameters in the protocol, it was decided that there will also be a column for free text to note down comments or observations that do not fit under any of the parameters.
Results

Description of the parameters

The selected parameters are presented in Table 1 according to the 12 welfare criteria. A full explanation of each parameter and its scoring scale, with references if available, is presented in Appendix 1 (see online at http://lan.sagepub.com). The explanation is presented in the form of a set of instructions. Each parameter is explained in a standard format: name, brief description, the different levels of the score, and comments including references to previous studies and especially information on the reliability of the parameter.

Choice of unit of measurement and scales in our protocol

Some parameters have been published with existing validated scales, like body condition score,6 nest building performance7 and facial expressions of pain8 and so these scales were also used in our protocol. For parameters that were taken from existing protocols (although not necessarily validated) we also used the scales from those protocols. Several parameters were given a ‘Yes/No’ scale in our protocol, e.g. ‘fur and whisker trimming’, ‘bite wounds’, ‘distended abdomen’ and ‘ocular/nasal discharge’. This was because, as stated above, the emphasis of this protocol is to identify whether an indicator of poor animal welfare was present or not. The scale for ‘ease of handling’ was converted from an assessment of handlers5 to an animal-based measure, and then further modified following a pilot study. The parameter ‘approaching hand in cage’ was developed by combining what is proposed for farm animals in the Welfare Quality8 protocols and the findings of Hurst and West27 on mice. The scale (i.e. distance from hand) was adapted to fit a standard sized mouse cage and the time allowed was reduced (from 60 s to 10 s) to be feasible as one parameter in the entire protocol.

The parameters ‘nest building performance’, ‘blood stains in the cage’ and ‘urine and faeces’ can only be measured at cage level, although this would be an individual measure if animals are singly-housed. Stereotypies are measured at cage level, although this measure is based on seeing individual animals performing them. The parameter ‘whiskers and/or fur trimming’ is measured at the individual level, but it is the receiver of the behaviour, not the performer, who is actually identified. The same principle applies to bite wounds. Determining the identity and number of individuals performing stereotyped behaviour, barbering or aggression was considered to be too time-consuming within the scope of this protocol. In addition, we regarded their mere presence in a cage as ‘bad enough’ and therefore did not regard it as necessary to record how many individuals were performing the behaviour. All other measures were recorded at the individual level, since they were relevant for the individual’s welfare and since it was possible to score each individual during the cage cleaning procedure.

Discussion

The results of this paper show that it is possible to identify animal-based measures for the assessment of laboratory mouse welfare based on the 12 welfare criteria from the Welfare Quality8 protocols. We propose that this protocol could be used to assess the many different dimensions of animal welfare. This protocol is important because it assesses welfare in the home cage, where laboratory mice spend most of their time and where breeding animals spend their whole life. The housing and husbandry routines are therefore very important factors for their welfare. Findings might also be related to experimental procedures, but the intention is to record what is observed, no matter what the cause. Information about the model might then explain the scores the animals received and, although indicating reduced welfare, they might nevertheless be in accordance with what is expected for that animal model. The protocol developed here is feasible to use, because it is based on parameters that can be measured in the animal room, without extra equipment. Most measures can be incorporated into the cage changing procedure and can be performed within 1.5–1.8 min/mouse.8 Some of the proposed measures such as the parameters ‘ability to reach food hopper/water nipple’ and ‘rearing’ require more time, but it is believed that the information gained is worth the extra time. An assessment protocol needs to be quick, practical and easy to apply before it can be implemented on a large scale.16 Furthermore, the assessment can be performed by one person. In our study we used two people, to allow discussion regarding the protocol.8 Once the assessor is familiar with the protocol, we believe that he/she can do it alone during the cage changing routine. In the following sections we discuss the resulting protocol in terms of its validity, the use of animal-based measures, the question of integration of parameters, the need for additional animal-based measures and presenting results to stakeholders.

Validation and benchmarking

In this paper, the process by which the animal-based measures were selected are explained. Whenever possible already validated measures where the inter-observer reliability can be demonstrated were used; thus large
parts of the protocol can already be assumed to be valid. The next step in the validation process was to test the whole protocol at animal facilities, to analyse the inter-observer reliability of all measures, evaluate assessment scales, and assess their feasibility. This has been done at three animal facilities in Sweden. These results support our proposal that the protocol can be used for self-monitoring within facilities by benchmarking the findings and tracking them over time. If it is used for self-monitoring, then facilities can choose whether to use the entire protocol at every benchmarking interval or to record some parameters more frequently than others because they might be of extra interest for that particular facility. The results also support the proposal that the protocol could be used to compare facilities with each other, as inter-observer reliability for the scores is generally high. This could potentially be of interest for a large institute or university that might have different animal facilities spread over its campus since local husbandry routines can differ even within the same institute.

Animal-based measures

It is clear from the literature search that research on mouse welfare is not evenly distributed over all the different dimensions of animal welfare. Perhaps not surprisingly, there are many measures related to health but very few, if any, related to comfort in the home cage (e.g. ‘thermal comfort’), ‘ease of movement’ and ‘human–animal interaction’. The reason for mouse welfare research to focus on injuries and diseases could likely be related to the use of different animal models, as well as the relative ease by which these parameters can be measured. Thus, for the criteria ‘absence of injuries’ and ‘absence of diseases’, it was relatively easy to choose parameters that have the best combination of validity, reliability and feasibility. It was nevertheless difficult to choose criteria where there were few or no parameters, and clearly more work is needed to identify and develop animal-based measures related to the home cage environment or handling procedures.

Leach et al. stated that it is important to assess both resource-based and animal-based measures for an effective welfare assessment and to be able to correlate resources with animal welfare outcomes. We have based our protocol on the argument that while these resource-based parameters are important as inputs and as risk factors, they do not need to be assessed on a regular basis. Instead, the emphasis is put on assessing the animals’ response to resources and management, i.e. the animal welfare outcome. This is in keeping with the statement by Whay et al., which states that the animals’ welfare state can be defined by ‘how well they are able to survive and remain fit within the particular constraints of the husbandry system in which they live’. Rather than collecting a large amount of information on resources and management routines (which should have been checked for compliance with legislation and good practice when first established), we suggest that a more time effective assessment alternative is to focus on what might be causing a less than perfect result for a particular animal-based measure. In order to then correctly interpret the results, background information on the strain of mice, age and sex is necessary. In this way the results of the protocol can be used to identify where efforts should be directed in order to improve animal welfare.

Integration of parameters or not?

The measures in the Welfare Quality protocols can be integrated into an overall assessment of a farm, that can be later communicated as animal welfare information to consumers buying the products. In the case of Welfare Quality this allows farms to be classified into one of four categories: not classified, acceptable, enhanced and excellent. Such a classification, or at least a threshold of what is acceptable or not, might also be beneficial for the proposed protocol for mice if it is to be used for official inspections. This would involve weighing different parameters and setting thresholds. A similar approach is suggested in the assessment of actual severity of experimental procedures, where the European Commission Expert Working Group on Severity Assessment advocates that everything the animal experiences during a procedure should be considered in the assessment. An equivalent process which rates the welfare parameters used in this protocol could be envisaged. But integrating scores from different parameters is a complicated procedure that requires advanced mathematical models, and ethical decisions have to be made on how to weigh different scores and how to combine different aspects of welfare to give a single value. For the purpose of assessing the welfare of laboratory mice in their home environment, the detailed scores in the parameters are of more interest (for benchmarking) than an overall integrated score. Indeed, details from different individual parameters that might be of interest will not be visible in an overall assessment.

In order to make the protocol user-friendly it is important to consider how to present the results. They could be presented per welfare parameter, per strain, per age group, etc. depending on the interest of that specific animal facility. It should be easy to backtrack from results to the parameters in question.
to identify reasons behind potential problems revealed in the results. Proper interventions can then be made and effects benchmarked over time. Within the scope of this study no specific effort has been directed towards the presentation of the results, but future work should be performed in order to make the protocol appealing and user-friendly. Further, the development of a management decision tool could be considered to help guide actions resulting from the assessment.

**Development of parameters**

We failed to find a useful, feasible animal-based measure for thermal comfort in adult mice. At present, thermal comfort is assessed only for pups (pups outside the nest). Behaviours reported to indicate responses to ambient temperatures are: thermotaxis (moving away from stressful temperatures), huddling and nest building in response to cold, and increased surface area exposure or fur licking in response to heat. Gordon et al. found that mice chose lower temperatures for their active period, that there was only a 1°C difference in choice of ambient temperature between singly-housed and group-housed mice, and that young mice (2 months of age) chose lower temperatures but compensated by being more active. Huddling does however seem to occur in a variety of ambient temperatures, e.g. Gordon et al. found that group-housed mice huddled in an ambient temperature of only 1°C lower than that preferred by singly-housed mice. Thus, unlike most other species, huddling is not a reliable indicator that a mouse is cold. Nest building can also be performed as protection and is regardless of reproductive status or sex. At present, the behaviours mentioned above need further investigation before it is clear which parameters should be incorporated into the protocol.

Since mice are generally housed in ambient temperatures below their thermoneutral zone, there have been fewer studies of whether mice might sometimes be kept in too warm an environment. In individually ventilated cages (IVCs), and especially in cages with high stocking densities, a lactating female risks becoming overheated since she is generating much heat as a by-product of milk production. Lactating female mice kept in IVCs (singly-housed) have been observed to rest outside the nest in a stretched-out position (E Spangenberg, personal observations) thereby increasing surface area exposure. Reliable and feasible parameters of heat stress are therefore also of interest, especially if mice kept in IVCs are to be assessed.

A positive emotional state in animals is one of the 12 welfare criteria and is also an area that is receiving increased attention within animal welfare science. Although it is believed that the traditional welfare parameters indicate an absence of poor welfare, they might not necessarily indicate that welfare is good. Parameters are needed to assess the positive side of animal welfare, but these are not easy to validate. There are many tests for assessing the emotional state in mice, but these generally measure negative states and require specific equipment. We propose using rearing behaviour when a mouse is initially put into its clean cage as an indicator of a positive emotional state. Rearing is considered to be an exploratory behaviour which, like play behaviour, is thought to be associated with a positive emotional state. However, it turned out to be impractical to score rearing in the current set-up of the protocol because as soon as a mouse was put into the new cage, it was intended that the assessor should start to observe the next mouse in the dirty cage. If the mouse did not immediately perform rearing in the new cage, the behaviour would be missed, and to wait for the mouse to perform the behaviour would prolong the observation time per mouse. For the parameter to be included in the protocol the assessor needs to watch the mouse closely when it is released into the new cage. So evaluating this parameter takes some additional time and may be included when a facility is especially interested in positive emotional states or for a particular group of mice or following a specific management modification. A possible alternative behaviour to observe in the home cage would be play behaviour, which is thought to be associated with a positive emotional state. However, play behaviour in mice is performed mainly by juveniles and the interactions are quick and brief. In addition, it would probably need to be observed during the dark period, when mice are active. Hence, at this stage it is not feasible to incorporate this behaviour into the protocol either, but it is a relevant measure of a positive emotional state (and one that can be measured in the home cage) and thus further studies in this area would be beneficial.

In summary, we have created a welfare assessment protocol for laboratory mice using only animal-based measures for evaluating animal welfare outcomes at an animal facility. We have also identified areas, such as thermal comfort behaviours and positive emotional states, where there are very few valid, reliable and feasible measures of mouse welfare and hence where more research is needed.

**Acknowledgement**

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**Ethical statement**

This manuscript is based on a literature search for parameters to be included in the protocol. Hence, no studies on animals were performed at this stage. It is however mentioned in the manuscript that the assessment protocol was tested at three animal facilities in Sweden. That part was approved by a Swedish regional committee for ethical approval of studies using animals for scientific purposes. Further, the assessment was performed on animals already available at the facilities visited and so was performed in line with the ‘reduction’ aspect of the 3Rs.

**References**

1. NC3R. What are the 3Rs? http://www.nc3rs.org.uk/page.asp?id=7 (cited 18 June 2013).


The effect of *Syphacia muris* on nutrient digestibility in laboratory rats

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Abstract

This study was carried out to investigate how pinworm infection in rats affects nutrient digestibility in the hosts. Twenty-four male outbred Wistar rats were randomly divided into two groups of 12 rats each. The rats from the first group (GI) were kept in cages with bedding containing pinworm eggs, and the second (control) group (GII) were kept in a separate room in clean, uncontaminated filter-top cages. The animals were put into individual metabolic cages later. Metabolic trials lasted five days and records of animal weight, food ingestion, and faecal weight were taken daily. Based on laboratory analysis of the feed and faecal nutrient content, digestibility values were determined. On day 15 of the experiment, the animals were euthanized. Although *Syphacia muris* were found in all rats from the GI group, animals exhibited no clinical signs. In our experiment, *S. muris* infection reduced the overall digestibility of all measured nutrients ($P < 0.01$). The most significant differences in digestibility were observed in the case of crude fibre and mineral matter ($P < 0.01$).

Keywords

laboratory rat, *Syphacia muris*, infection, nutrient, digestibility

Rats are the world’s most frequently used laboratory animals. They are used for scientific as well as for a variety of commercial purposes. They are easy to keep, and their breeding technology is very sophisticated. Nevertheless, rats suffer from many parasitic diseases, which can influence experimental results.

One of the most common parasites found in breeding rats is the pinworm. Due to their biology, direct development, short embryonic period, and incidence of autoinfection, pinworms are a very prolific group of parasites. In laboratory rats, the most commonly found pinworm species are *Syphacia muris* and *Aspiculuris tetraperta*.

Although pinworm parasites of laboratory rodents are considered to be relatively non-pathogenic and infections are generally regarded as symptomless, there have been reports of laboratory rodents with a variety of conditions such as intestinal impaction, intestinal intussusception, mucoid enteritis, necrosis in all layers of the intestinal wall, and rectal prolapse. These conditions are thought to be associated with heavy pinworm burdens.¹⁻⁶

One area in which rats are often utilized is feed testing. Rats are most commonly used in experiments designed to test the digestibility of individual nutrients in various feeds. However, it is very difficult to keep rats free of pinworm infection,⁷,⁸ and therefore, many of these experiments are conducted with infected animals.

The aim of this study is to investigate how pinworm infection affects nutrient digestibility. We monitored...
Materials and methods

Animals and procedure

Twenty-four male outbred Wistar rats were obtained from the specific pathogen-free (SPF) rat colony of Charles River Laboratories, Sulzfeld, Germany. The animals had an average weight of 89 g (4 weeks old). They were randomly divided into two groups of 12 rats each. The rats from the first (experimental) group (GI) were kept in the cages with bedding containing pinworm eggs for 10 days. Contamination was achieved by adding bedding from the cages of *S. muris* infected rats to the breeding containers. After this 10-day period, *Syphacia* eggs in all the animals of GI were detected using a cellophane-tape test. The second (control) group (GII) was kept in a separate room in clean, uncontaminated filter-top cages for 10 days; the beddings of GII group were thoroughly sterilized. The rooms were maintained at 22°C ± 2°C and 55 ± 5% relative humidity, with normal 12:12 h light–dark cycles and constant air circulation. All animals were monitored daily for the presence of pinworm eggs using the cellophane-tape test.

Thereafter, the animals were kept in individual metabolic cages (Tecniplast, Buguggiate, Italy) with normal 12:12 h light–dark cycles and constant air circulation. All animals were monitored daily for the presence of pinworm eggs using the cellophane-tape test.

Table 1. Composition of rat diet (ST-1 Bergman).

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>%</td>
</tr>
<tr>
<td>Ash</td>
<td>%</td>
</tr>
<tr>
<td>Dry matter</td>
<td>%</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>%</td>
</tr>
<tr>
<td>Nitrogen-free extract</td>
<td>%</td>
</tr>
<tr>
<td>Fat</td>
<td>g/100 g</td>
</tr>
<tr>
<td>Calcium [Ca]</td>
<td>mg/kg</td>
</tr>
<tr>
<td>Potassium [K]</td>
<td>mg/kg</td>
</tr>
<tr>
<td>Magnesium [Mg]</td>
<td>mg/kg</td>
</tr>
<tr>
<td>Sodium [Na]</td>
<td>mg/kg</td>
</tr>
<tr>
<td>Phosphorus [P]</td>
<td>mg/kg</td>
</tr>
</tbody>
</table>

Metabolic trials lasted five days, and records of animal weight, food ingestion, and faecal weight were taken daily. Daily faecal outputs were collected in plastic bags, weighed, thoroughly mixed and stored at −20°C for later analysis.

From these measured values, we calculated daily weight gain, feed intake and feed conversion. Based on laboratory analysis of the nutrient content of feed and faeces, digestibility values were determined.

On day 15 of the experiment, the animals were euthanized using an intravenous administration of T-61 solution (Intervet, Boxmeer, The Netherlands). The viscera (stomach, small intestine, colon and caecum) of each rat were collected and processed for worm recovery, enumeration and identification.

All of the experimental procedures were conducted in accordance with Czech legislation (section 29 of Act No 246/1992 Coll., on the protection of animals against cruelty, as amended by Act No 77/2004 Coll. and Directive 2010/63/EU on the protection of animals used for scientific purposes and the guidelines and recommendations of the Federation of European Laboratory Animal Science Associations).

Laboratory analysis

The food and faecal samples were lyophilized (LP3 lyophilizer; Jouan, France) and grounded (Cyclotec 1093, Tecator, FOSS, Hillerød, Denmark) in order to pass through a one-millimetre stainless steel screen. The feed and faecal dry matter contents were determined at a temperature of 103°C (UFB500, Memmert). The Kjeldahl method (Kjeltec 2400, Foss) was used to determine crude protein (CP) levels, and crude fibre was measured using the Henneberg and Stohmann method (Coex 106, VELP Scientifica, Usmate, Italy). Fat digestibility was analysed with the help of the Soxhlet method (SER 146/8, VELP Scientifica). This method is based on the principle of extraction with an organic solvent (petroleum ether). During this extraction the results include fat and any other substances which are readily soluble. The content of other substances, such as carotenoids, chlorophylls, cholesterol and fat-soluble vitamins, were not taken into account. Gross energy was measured using an LS10-A calorimeter (Laget, Prague, Czech Republic), and ash content (mineral matter) was determined at 550°C (LH 15/13; LAC, Rajhrad, Czech Republic). The ash samples were incinerated, then boiled in 6 M hydrochloric acid and the insoluble residue was filtered through an ashless filter, which was dried and burned. Analyses were performed according to EC No. 156/2006 modified to a specific device.

Values of nitrogen-free extract (NFE) were calculated as follows: 100−(% moisture + % protein + %
fibre + % ash + % fat). Nutrient digestibility was defined as the difference between food intake and faecal excretion expressed as a percentage of the intake: 100 - \[\frac{[(\text{feed insoluble ash} \times \text{faecal nutrient}) - \text{excretion})/\text{intake}] \times 100}{\text{faeces insoluble ash} \times \text{feed nutrient}}\].

**Data analysis**

The mean and standard deviation for each group were calculated and compared using analysis of variance (ANOVA), with the Tukey–Kramer test to evaluate differences among the groups.

**Results**

*Syphacia muris* were found in all the rats from the GI group (Table 2); GII rats were free of these parasites. All the rats in the experiments remained in good health, with no clinical signs or visible changes in the mucosa, no alterations in the structure of faeces, and no signs of diarrhoea. Therefore, pinworm infection could be generally regarded as subclinical. In the current experiment, *S. muris* infection reduced the overall digestibility of all measured nutrients \((P < 0.01)\) (Table 3). The feed intake of the healthy animals was higher than that of the infected animals, and the difference was statistically significant (Table 4).

**Table 2.** Number of *Syphacia muris* individuals in the rats of the GI and GII groups.

<table>
<thead>
<tr>
<th>GII</th>
<th>Adults</th>
<th>Larvae</th>
<th>Male</th>
<th>Female</th>
<th>GII</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>450</td>
<td>78</td>
<td>130</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1080</td>
<td>73</td>
<td>71</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>740</td>
<td>47</td>
<td>53</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>720</td>
<td>263</td>
<td>215</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>350</td>
<td>32</td>
<td>22</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>680</td>
<td>19</td>
<td>21</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>400</td>
<td>73</td>
<td>44</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>590</td>
<td>127</td>
<td>109</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>280</td>
<td>95</td>
<td>81</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>1220</td>
<td>96</td>
<td>89</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>670</td>
<td>223</td>
<td>187</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>690</td>
<td>128</td>
<td>187</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

GI: Rats from the first (experimental) group were kept in the cages with bedding containing pinworm eggs for 10 days. GII: Control group rats were kept in a separate room in clean, uncontaminated filter-top cages for 10 days.

The most significant differences in digestibility were observed in the cases of crude fibre and mineral matter \((P < 0.01)\). Crude fibre digestibility in the uninfected rats was very high \((36.95 \pm 10.28\%)\), whereas crude fibre digestibility values in the animals with pinworms averaged \(23.88 \pm 3.03\%). Similarly, mineral matter retention was significantly lower in the infected animals \((59.46 \pm 4.62\%)\) than in the control rats \((82.95 \pm 1.88\%).\) The values of nutrient digestibility are shown in Table 3 and growth parameters are presented in Table 4.

**Discussion**

Rats are among the most important laboratory animals. They are used for a variety of experiments, including metabolic trials and food testing. Unfortunately, pinworm infection is not taken into account in many of these assays. Although these parasites are considered non-pathogenic, pinworms definitely affect their hosts in a variety of ways. Wagner

**Table 3.** Average values of nutrient digestibility of monitored rat groups [%].

<table>
<thead>
<tr>
<th></th>
<th>Uninfected</th>
<th>Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash (mineral matter) digestibility</td>
<td>82.95 ± 1.88</td>
<td>59.46 ± 4.62*</td>
</tr>
<tr>
<td>Crude protein digestibility</td>
<td>93.94 ± 1.00</td>
<td>87.19 ± 1.76*</td>
</tr>
<tr>
<td>Fat extract digestibility</td>
<td>94.79 ± 1.51</td>
<td>86.53 ± 1.86*</td>
</tr>
<tr>
<td>Crude fibre digestibility</td>
<td>36.95 ± 10.28</td>
<td>23.88 ± 3.03*</td>
</tr>
<tr>
<td>Organic matter digestibility</td>
<td>94.07 ± 1.01</td>
<td>85.13 ± 1.64*</td>
</tr>
<tr>
<td>Nitrogen-free extract digestibility</td>
<td>95.54 ± 1.82</td>
<td>87.58 ± 1.52*</td>
</tr>
</tbody>
</table>

*Significantly different from control group \((P < 0.01)\).

**Table 4.** Growth parameters of monitored rat groups.

<table>
<thead>
<tr>
<th></th>
<th>Uninfected</th>
<th>Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial weight [g]</td>
<td>224.08 ± 10.40</td>
<td>198.37 ± 6.20†</td>
</tr>
<tr>
<td>End weight [g]</td>
<td>290.96 ± 12.72</td>
<td>237.95 ± 11.14†</td>
</tr>
<tr>
<td>Daily gain [g]</td>
<td>16.72 ± 1.76</td>
<td>9.90 ± 1.42†</td>
</tr>
<tr>
<td>Feed conversion gain [g*]</td>
<td>1.75 ± 0.17</td>
<td>2.34 ± 0.21†</td>
</tr>
<tr>
<td>Daily feed intake [g]</td>
<td>29.05 ± 0.99</td>
<td>22.92 ± 1.75†</td>
</tr>
</tbody>
</table>

*Feed intake [g] per gram weight gain.
†Significantly different from control group \((P < 0.01)\).
reported that uninfected rats grew faster and attained weights (at 6 weeks) that were, on average, 12% higher than those of their infected counterparts. The competition for and utilization of the host nutrients are likely explanations for growth depression.10 Pinworms cause changes in haemopoiesis,11 affect lymphocyte proliferation12 and reduce water and electrolyte transport in the intestine.13

The results of this study show that pinworms also negatively affect the digestibility of all evaluated nutrients. We compared parasitized rats to non-parasitized rats given access to the same amounts of food, so that the consequences of parasitism on food utilization could be directly quantified and evaluated. Statistically significant differences were observed between groups GI and GII with respect to digestibility of all tested nutrients.

The effects were most marked for crude fibre and mineral ash. This is a consequence of the fact that pinworms are located primarily in the caecum, where fermentable polysaccharides act as an energy source for microorganisms, and where mineral absorption takes place. Similar results have been noted in pigs infected with the nematode *Oesophagostomum dentatum*, which is localized in the colon; and all of these digestibility coefficients measured were affected by this parasite. However, the effects were most marked for crude fibre and mineral ash.14 Similar changes were also reported in studies by Kaarma.15 Munger and Slichter16 reported significantly low dry matter digestibility in kangaroo rats (*Dipodomys microps*) with *Trichuris dipodomys* infection.

The role of the large intestine in digestion has not been fully documented. In rats this is mainly where bacteria degrade the fermentable part of fibre to short-chain fatty acids, which are sources of energy and are precursors in the synthesis of glucose and body fat, and which also play an important role in various physiological functions.17 The main site of fibre degradation in rats is the caecum, and in this study, 98% of all pinworm populations were found in this region. Although pinworms did not cause visible clinical symptoms or changes in the mucosa, caecum function was likely to have been affected. At the end of the experiment, we found 404 to 1405 individual *S. muris* nematodes in one rat!

Most studies dealing with nutrient digestibility in relation to parasitic infection have been carried out on ruminants with nematodes, which are located in the abomasum and small intestine.18–23 The interaction between intestinal helminth infection and nutrition has been reviewed by Poppi et al.,24 Van Houtert and Sykes,25 Coop and Holmes,26 Coop and Kyriazakis,27 Petkevičius,28 and Holmes et al.29 In general, gastrointestinal nematodes reduce nutrient availability in the host through reductions in both voluntary feed intake and/or absorbed nutrient efficiency. The degree to which these two mechanisms impair production is, to some extent, dependent on the species of parasite and its location in the gastrointestinal tract.27,30

The key feature of gastrointestinal nematode infection is an increased loss of endogenous protein to the gastrointestinal tract,31–34 partly as a result of plasma protein leakage, increased mucoprotein production and sloughing of epithelial cells in the alimentary tract.32 Poppi et al.31 and Kimambo et al.35 have suggested that the amount of non-reabsorbed endogenous nitrogen that leaves the terminal ileum may be considerable (up to 4–5 g of nitrogen per day). In this study, high protein digestibility was observed in both rat groups. The main reason for this was the feed composition, which contained large proportions of fish; as a result, both groups of rats grew very quickly. However, there was also a statistically significant difference in CP digestibility in the control group. This may have been due to limited caecum function as well as to reduced absorption or utilization of ammonium nitrogen in other forms, which also takes place in the caecum.36

The most significant effect that gastrointestinal parasitism has on its host is voluntary feed intake depression.22,23,37,38 Large acute infections result in significant decreases in the feed intake of parasitized animals;39 however, a degree of inappetence is present even in subclinical infections.28 Several hypotheses have been postulated for this reduction in voluntary feed intake, such as alterations in amino acid availability, changes in flow rates and pH of digests, alterations in gut peptides and hormones, and direct neural effects on the central nervous system.28,40 Even in this current study, the feed intake of healthy animals was higher than that of infected animals, and the difference was statistically significant.

Recently, the influence of nutrition on gastrointestinal parasites has been examined from different perspectives. Some studies show that reductions in voluntary feed intake, as measured by a depression of dry matter intake, are observed only during primary infection.22,23 Other studies have shown that adding protein to the animal diet improves resistance and resilience to several nematode infections.21,28,41 In recent times, the influence of host nutrition on helminth populations has also received attention, and much information is available.28,42–47 Gastrointestinal helminths have very specific physicochemical requirements of their host gut environment, and nutritionally mediated changes may have a direct influence on parasite populations.48

The possible effects that varying levels of dietary fibre intake have on the occurrence of endoparasitic infection in pigs was reported in the study by Pearce,49 in which the use of grower diets high in
non-starch polysaccharides was associated with an increased risk of Trichuris infection.

In our study, *S. muris* infection caused no visible signs; no visible changes in the mucosa, no alteration in faecal structure, and no diarrhoea. However, pinworms definitely affect their hosts in different ways. Pinworms alter the gastrointestinal tract environment as well as nutrient digestibility, and they most likely also affect the bacterial community structure, including possible secondary bacterial infections. A secondary bacterial infection would likely lead to impaired absorption and an increase in the flow of materials. Pattison et al. have reported that a high flow rate of digesta in the large intestine would reduce the duration of bacterial action on dietary fibre, thus accounting for a reduction in digestibility.

In conclusion, rats infected with *S. muris* are not suitable for experiments designed to test nutrient digestibility in various feeds, because these parasites significantly reduce nutrient digestibility of individual nutrients (*P* < 0.01). It is difficult to understand the mechanisms involved in these reductions, but it has been suggested that subclinical parasitism may affect the nutrition of the host, particularly through reduced absorption, an increased flow rate of digesta, and reduced enzyme activity. Further detailed investigations would be beneficial.

**Acknowledgement**
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**Declaration of conflicting interests**
On behalf of all authors the corresponding author states that there is no conflict of interest.

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**Compliance with ethical standards**
All experiments with laboratory animals were conducted in compliance with the current laws of the Czech Republic Act No. 246/1992 coll. on the protection of animals against cruelty and EC Directive 86/609/EEC.

**References**


Effective cryopreservation of golden Syrian hamster embryos by open pulled straw vitrification

Z Fan¹, Q Meng¹, TD Bunch¹, KL White¹ and Z Wang¹,²

Abstract
Golden Syrian hamster embryos are difficult to cryopreserve due to their high sensitivity to cryoprotectants and in vitro handling. The objective of this study is to develop a robust open pulled straw (OPS) vitrification technique for cryopreserving hamster embryos at various developmental stages. We first systematically tested the concentrations of cryoprotectants and the exposure times of two-cell embryos to various vitrification solutions. We identified pretreatment of two-cell embryos with 10% (v/v) ethylene glycol (EG) + 10% (v/v) dimethylsulfoxide (DMSO) for 30 s followed by exposure in the vitrification solution, EDFS30 [containing 15% EG + 15% DMSO], for 30 s before plunging into liquid nitrogen (two-step exposure method) as the optimal OPS vitrification protocol. We then investigated the resourcefulness of this protocol for vitrifying hamster embryos at different developmental stages. The results showed that high blastocyst rates from embryos vitrified at two-cell, four-cell, eight-cell, or morula stage (62%, 78%, 80%, or 72%, respectively), but not those verified at pronuclear (0%) or blastocyst stage (24%; \( P < 0.05 \)), were achieved by this protocol. When embryos vitrified at the two-cell stage were recovered and then directly transferred to recipient females, 29% of them developed to term, a development rate not significantly different (\( P > 0.05 \)) from the 40% birth rate of the unvitrified controls. In conclusion, we have developed an effective two-step OPS vitrification protocol for hamster embryos.

Keywords
hamster, embryo, OPS vitrification

The golden Syrian hamster (Mesocricetus auratus) has been used as an animal model in many research areas, including virology,¹⁻³ endocrinology,⁴ metabolism,⁵⁻⁶ diabetes,⁷ cancer,⁸⁻⁹ and cardiovascular diseases.¹⁰ With the recent completion of the draft assembly of its genome by the Genome 10 K Projects at the National Human Genome Research Institute (NHGRI: www.genome.gov), the application of hamsters as animal models for biomedical research will certainly increase. Furthermore, the recent technology advancement achieved by us in employing the Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR associated protein 9 (CRISPR/Cas9) system to efficiently conduct gene targeting in hamsters¹¹ has overcome another major barrier set up by the lack of gene targeting tools in this species. Other researchers are also making progress in developing tools to genetically modify the hamster genome.¹² Such technology advancement will inevitably promote the production and application of genetically-engineered hamsters in the near future. Since the maintenance of transgenic and gene-modified lines is costly and takes up space in animal facilities, cryopreservation of hamster embryos will be widely used and may become a routine in the laboratory. However, a simple and reliable cryopreservation technique for cryopreserving hamster embryos is currently lacking.

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Email: zonda.wang@usu.edu
In 1985, Ridha and Dukelow\textsuperscript{13} reported the first cryopreservation of hamster preimplantation embryos with slow freezing, but failed to produce live offspring. It is difficult to cryopreserve hamster embryos for several reasons. First, the widely used vitrification cryoprotectants, dimethylsulfoxide (DMSO) and ethylene glycol (EG), have detrimental effects on the in vitro development of earlier stage hamster embryos.\textsuperscript{14} Second, hamster embryos are extremely sensitive to environmental factors, including light,\textsuperscript{15} and chemicals,\textsuperscript{16,17} as well as the duration and temperature of culture medium equilibration,\textsuperscript{18} which are normally tolerated well by embryos from other species. In agreement with these, Mochida et al. showed that only eight-cell stage hamster embryos could be cryopreserved and that the cryopreserved embryos had very poor chances of development into blastocysts after being frozen and thawed.\textsuperscript{14} So far, only one group has reported successful vitrification of hamster embryos with a cryoloop method and, with a relatively lower level of success, with an open pulled straw (OPS) method.\textsuperscript{19} In this study, only one-cell and two-cell stage hamster embryos were used.\textsuperscript{19} While the cryoloop technique seems to be adequate for cryopreserving one-cell and two-cell stage hamster embryos, it requires direct contact between embryos and liquid nitrogen (LN\textsubscript{2}),\textsuperscript{20} which raises concerns about the risks of contaminating embryos with infectious pathogens from LN\textsubscript{2}.\textsuperscript{21}

OPS vitrification was developed to cryopreserve bovine oocytes and in vitro produced embryos at various developmental stages by Vajta et al. in 1998.\textsuperscript{22} Subsequently, studies have reported on the successful application of OPS techniques in cryopreserving mouse,\textsuperscript{23,24} rat,\textsuperscript{25} rabbit,\textsuperscript{26} sheep,\textsuperscript{27} goat,\textsuperscript{28} and porcine\textsuperscript{29} embryos. Both OPS and cryoloop vitrification, using a minimum volume of cryoprotectants, belong to the third generation of cryopreservation technology,\textsuperscript{30} relative to the slow freezing and conventional vitrification methods. The aims in using the OPS method are to prevent ice formation and reduce chilling injury, toxicity of cryoprotectants and osmotic damage to embryos through improving cooling and warming rates.\textsuperscript{22} Many reports have shown that the OPS method is superior to slow freezing with regard to survival, in vitro and in vivo development of cryopreserved embryos.\textsuperscript{26,31,32} Moreover, by applying a modified procedure for embryo loading, the OPS method can be performed under sterile conditions and can comply with sanitary regulations.\textsuperscript{33,34}

The present study was designed to establish and optimize an OPS method for hamster embryo cryopreservation and to investigate the effectiveness of such a method in cryopreserving embryos at different developmental stages.

**Materials and methods**

**Animals**

Golden Syrian hamsters used for embryo collection were bred in-house by using founder animals purchased from Charles River, Kingston, NY, USA (LVG Golden Syrian Hamster, Strain Code: 049). Black Syrian hamsters used as recipients for embryo transfer were acquired from a breeding colony established in our laboratory. All hamsters were raised and maintained in an air-conditioned room with a 14:10 light–dark cycle (lights on from 06:00 h). The hamsters used were healthy and free of rodent pathogens, as demonstrated by routine monitoring of sentinel animals housed in the same room. In total, 90 golden Syrian females were used as embryo donors and 25 black females as recipients for embryo transfer. The experiments were conducted in accordance with the guidelines of the Laboratory Animal Research Center at the Utah State University and approved by the Institutional Animal Care and Use Committee (IACUC Protocol: 2091).

**Reagents and media**

All reagents were purchased from Sigma-Aldrich (St Louis, MO, USA) unless otherwise stated. Hamster embryos were cultured in HECM-9\textsuperscript{16} supplemented with 0.5 mg/mL human serum albumin (A1653). The Hapes-buffered HECM-9 (HHECM-9) was used for in vitro manipulation of embryos, which contained 20 mmol/L HEPES and 5 mmol/L NaHCO\textsubscript{3}. The pre-treatment solution for embryo vitrification consisted of 10% (v/v) EG+10% (v/v) DMSO in HHECM-9. Four vitrification solutions consisted of 12.5% EG+12.5% DMSO, 15% EG+15% DMSO, 17.5% EG+17.5% DMSO, and 20% EG+20% DMSO in HHECM-9 supplemented with 300 mg/mL Ficoll and 171.2 mg/mL sucrose, defined hereafter as EDFS25, EDFS30, EDFS35, and EDFS40, respectively.

**Embryo collection**

Hamster embryos were collected from 2–3-month-old, cycling golden hamsters. Hamsters were induced to superovulate by intraperitoneal injection of pregnant mare’s serum gonadotrophin (PMSG, G4877) with weight-dependent dosages (10 IU if <90 g, 15 IU if 91–115 g, 20 IU if 116–140 g, and 25 IU if >141 g)\textsuperscript{16} at 09:00 h on the day of post-estrous discharge (day 1 of cycle).\textsuperscript{35} Females were mated to fertile males at 19:00 h on day 4. One-cell embryos at the pronuclear (PN) stage were collected from oviducts at 15:00 h on day 1, two-cell at 08:00 h and four-cell at 22:00 h from oviducts on day 2, eight-cell at 16:00 h and morula at 20:00 h from uteri at day 3, and blastocysts at 08:00 h...
from uteri at day 4, by flushing with HHECM-9 pre-equilibrated in a CO₂ incubator (37.5°C, 10% CO₂, 5% O₂, and 85% N₂). Shortly after collection, embryos at different stages were either cultured to blastocysts or subjected to vitrification. The in vitro manipulation of embryos was performed in a dark room with a small incandescent lamp, and red filters were used on the microscope light source, as described by Yamauchi et al.26

**OPS vitrification**

The OPS was made using the method described by Vajta et al.22 with some modifications. Briefly, a 0.25 mL straw (IVM, L’Aigle, France) was heat-softened over a small alcohol burner, pulled manually and cut at the tapered end with a blade. The inner diameter of the tip was 0.20–0.25 mm and the wall thickness was approximately 0.05 mm. Embryo freezing and thawing were performed on a 37.5°C hot plate, and the ambient temperature was maintained at 25 ± 1°C. Embryos were pretreated in 10% EG + 10% DMSO for 30 s, and then transferred to the EDFS solution and held for 30 s, 60 s, or 120 s, respectively. After that, embryos were picked up by the narrow end of the OPS. The OPS loaded with embryos was immediately plunged into LN₂. Approximately 10 embryos were loaded into each OPS and stored in LN₂ for at least 48 h. When thawing, the OPS was taken out of the LN₂, and the narrow tip was immersed immediately in 1 mL of warmed (37.5°C) 0.5 M sucrose. Embryos were expelled from the OPS and transferred into another drop of the same solution for 5 min to dilute cryoprotectants. After thawing, embryos were either cultured to blastocysts or transferred to recipients after 0.5 h recovery in HECM-9.

**Embryo transfer**

Viability of vitrified two-cell embryos was assessed by transfer to recipient females. Black Syrian females, naturally mated with black males, one day before, were used as recipients, and the color of hair was used to distinguish between pups from transferred embryos and from natural mating. The surgery of embryo transfer was based on the procedure described by Farrell and Bavister37 with some modification. The recipients were induced to anesthesia by intraperitoneal injection of 100 mg/kg ketamine and 10 mg/kg xylazine. Approximately 2 cm incisions were made with fine dissection scissors on the left and right paralumbar sites. After pulling out the ovary, oviduct, and part of the uterine horn, the oviduct was positioned through changing the positions of the serrefine clamp and the hammer. The wall of the oviduct between the infundibulum and ampulla was dissected with micro-spring scissors. The tip of the capillary containing the embryos was inserted into the cut, and then pushed further towards the ampulla. The embryos were expelled into the ampulla with a minimum amount of collection medium. Eight vitrified-thawed embryos with normal morphology (with 2 even fully-expanded blastomeres) and from natural mating. The surgery of embryo transfer was analyzed using arcsine transformation, followed by one-way or two-way analysis of variance (ANOVA), as appropriate. A P value of <0.05 for effects of factors (media, programs, developmental stages and vitrification) or interactions between factors (developmental stages and vitrification) was considered significant. When any of the factors had significant effects on the parameters, a post hoc procedure with least significant difference (LSD) test was applied to compare means.
difference (LSD) tests was used for multiple and pairwise comparisons between groups. Means from differential staining were compared by the independent-samples r-test. All data were analyzed with a computer program, SPSS for Windows (Version 16.0; SPSS Inc, Chicago, IL, USA).

**Results**

*In vitro development of two-cell embryos vitrified using different cryoprotectant solutions*

Embryos were pretreated with 10% EG + 10% DMSO for 30 s, followed by 30 s treatment in four vitrification media containing different concentrations of cryoprotectants before being plunged into LN2. As shown in Table 1, all embryos were recovered after vitrification with normal morphology but showed different developmental potential among the groups treated with different cryoprotectant solutions. The in vitro development of embryos vitrified by EDFS25 or EDFS40 arrested before developing to morula or four-cell stage, respectively. On the other hand, when EDFS30 or EDFS35 was used, vitrified embryos developed to the blastocyst stage. Moreover, the blastocyst rate of embryos vitrified by EDFS30 (53%) was significantly higher ($P < 0.05$) than that by EDFS35 (20%), but lower ($P < 0.05$) than that of untreated controls (78%).

In *in vitro development of two-cell embryos vitrified using different cryopreservation programs*

Based on the results in Table 1, we further optimized vitrification conditions by using EDFS30 in different vitrification programs which were designated as Programs A, B, C, and D (Table 2). After thawing, all embryos cryopreserved from each of the programs recovered with normal morphology. The highest blastocyst rate (62%) was achieved from embryos vitrified with Program B, in which embryos were pretreated in 10% EG + 10% DMSO for 30 s, followed by holding in EDFS30 for 30 s. The blastocyst rate of Program B was similar ($P > 0.05$) to that of Program A (58%, without pretreatment) and Program C (47%, holding in

**Table 1.** Effect of the concentration of cryoprotectants on in vitro development of vitrified two-cell hamster embryos.

<table>
<thead>
<tr>
<th>Concentration of cryoprotectant</th>
<th>No. of embryos treated*</th>
<th>No. (% ± SEM) of embryos recovered after vitrification†</th>
<th>No. (% ± SEM) of embryos developing to</th>
<th>≥ 4-cell</th>
<th>Morula</th>
<th>Blastocyst</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>31</td>
<td>–</td>
<td>30 (97 ± 3)a</td>
<td>30 (97 ± 3)a</td>
<td>24 (78 ± 12)a</td>
<td></td>
</tr>
<tr>
<td>EDFS25</td>
<td>28</td>
<td>28 (100 ± 0)a</td>
<td>6 (22 ± 13)d</td>
<td>0 (0 ± 0)d</td>
<td>0 (0 ± 0)d</td>
<td></td>
</tr>
<tr>
<td>EDFS30</td>
<td>32</td>
<td>32 (100 ± 0)a</td>
<td>26 (81 ± 6)b</td>
<td>23 (72 ± 6)b</td>
<td>17 (53 ± 7)b</td>
<td></td>
</tr>
<tr>
<td>EDFS35</td>
<td>30</td>
<td>30 (100 ± 0)a</td>
<td>17 (57 ± 3)b,c</td>
<td>9 (30 ± 6)c</td>
<td>6 (20 ± 6)c</td>
<td></td>
</tr>
<tr>
<td>EDFS40</td>
<td>38</td>
<td>38 (100 ± 0)a</td>
<td>0 (0 ± 0)e</td>
<td>0 (0 ± 0)d</td>
<td>0 (0 ± 0)d</td>
<td></td>
</tr>
</tbody>
</table>

*Total number of embryos in each group derived from three replicates; within each replicate, 8–15 embryos were treated.
†Means and SEMs were calculated arithmetically and analyzed by one-way analysis of variance (ANOVA).

a,b,c,d,eWithin a column, values with no common letters are significantly different ($P < 0.05$). –: not applicable.

**Table 2.** Effect of different vitrification programs on in vitro development of vitrified two-cell hamster embryos.

<table>
<thead>
<tr>
<th>Program</th>
<th>Pretreatment time (s) / Treatment time (s)</th>
<th>No. of embryos vitrified*</th>
<th>No. (% ± SEM) of embryos recovered after vitrification†</th>
<th>≥ 4-cell</th>
<th>Morula</th>
<th>Blastocyst</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>–/30</td>
<td>31</td>
<td>31 (100±0)a</td>
<td>30 (97 ± 3)a</td>
<td>28 (91 ± 5)a</td>
<td>18 (58 ± 11)a</td>
</tr>
<tr>
<td>B</td>
<td>30/30</td>
<td>35</td>
<td>35 (100±0)a</td>
<td>30 (86 ± 3)b,a</td>
<td>26 (75 ± 4)a</td>
<td>22 (62 ± 4)a</td>
</tr>
<tr>
<td>C</td>
<td>30/60</td>
<td>33</td>
<td>33 (100±0)a</td>
<td>27 (81 ± 10)b,a</td>
<td>24 (73 ± 9)a</td>
<td>15 (47 ± 11)b,a</td>
</tr>
<tr>
<td>D</td>
<td>30/120</td>
<td>33</td>
<td>33 (100±0)a</td>
<td>18 (51 ± 21)b</td>
<td>13 (37 ± 13)b</td>
<td>9 (26 ± 8)b</td>
</tr>
</tbody>
</table>

*Total number of embryos was derived from three replicates in each program; within each replicate, 8–15 embryos were treated.
†Means and SEMs were calculated arithmetically and the means were analyzed by one-way analysis of variance (ANOVA).

a,bWithin a column, values with no common letters are significantly different ($P < 0.05$). –: the embryos were vitrified without pretreatment.
EDFS30 for 60 s). When the exposure time in EDFS30 was extended to 120 s (Program D), the blastocyst rate decreased to 26% and was significantly lower ($P < 0.05$) than that which was observed in Program B.

**In vitro development of embryos vitrified at different developmental stages**

After establishing Program B to be the most effective vitrification program, we investigated the development potential of embryos vitrified at different developmental stages utilizing this program. Figure 1A shows the in vitro development of the embryos vitrified at different developmental stages. Pairwise comparisons showed no significant differences ($P > 0.05$) in blastocyst rates among vitrified two-cell (62%), four-cell (78%), eight-cell (80%), or morula (72%) embryos, but the blastocyst rates in all of these groups were significantly higher ($P < 0.01$) than that of the vitrified blastocysts (24%). Most dramatically, the in vitro development of vitrified PN embryos was arrested at the two-cell stage, even though 51% of PN control embryos developed to blastocysts. During the experiments, we also monitored the development of unvitrified control embryos isolated at the four-cell, eight-cell and morula stages. As shown in Figure 1A, embryos isolated at the four-cell, eight-cell and morula stages had high developmental ability with blastocyst rates of 91%, 92%, and 96%, respectively (with no significant differences among them); however, the blastocyst rates of embryos isolated at PN or the two-cell stage (51% or 79%, respectively) were significantly lower ($P < 0.05$) than embryos isolated at the four-cell, eight-cell and morula stages. When vitrified and unvitrified controls were compared at each of the corresponding developmental stages, no significant differences ($P > 0.05$) were observed between vitrified and control embryos at the two-cell, four-cell, or eight-cell stage, except for the embryos vitrified at morula or blastocyst stage (Figure 1A).

**Differential staining of blastocysts derived from vitrified two-cell embryos**

Significant decreases ($P < 0.05$) in total cell (48.7 ± 3.3 vs. 60.1 ± 3.0) and trophectoderm (TE) cell numbers

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**Figure 1.** (A) In vitro development of hamster embryos at different developmental stages vitrified using EDFS30 media. The blastocyst rate was expressed as mean (%) + SEM, and the means were analyzed by two-way analysis of variance (ANOVA). PN, 2 C, 4 C, 8 C, M, and B: pronuclear, two-cell, four-cell, eight-cell, morulae, and blastocyst stage, respectively. a,bWithin each developmental stage, values with no common letters are significantly different ($P < 0.05$). A–DAmong vitrified or control groups, values with no common letters are significantly different ($P < 0.05$). (B) Differential staining of a hamster blastocyst derived from in vitro culture of vitrified/thawed two-cell embryos. (a) Inner cell mass (ICM) cells identified by their red fluorescence; (b) Total cells identified by their blue fluorescence; (c) A merged photograph shows ICM and trophectoderm (TE) cells. Scale bar equals 60 μm.
(34.5 ± 2.5 vs. 43.9 ± 2.4) were observed in blastocysts from vitrified two-cell embryos compared with control embryos (Table 3). The mean inner cell mass (ICM) of vitrified embryos was slightly, but not significantly, lower (P > 0.05) than that of controls (14.2 ± 1.2 vs. 16.2 ± 1.4, Table 3). No significant differences (P > 0.05) were found in the ratios of ICM to total cell number between vitrified and control embryos (29.3 ± 1.3% vs. 26.8 ± 1.8%, Table 3). Figure 1B shows representative photographs for differential staining of a blastocyst that was derived from in vitro culture of a vitrified two-cell embryo.

### Embryo transfer

Four vitrified two-cell embryos that appeared morphologically normal after thawing were transferred to each oviduct of the recipients. Of the seven transfers performed, six (86%) resulted in pregnancies and the production of normal offspring. Figure 2 shows a surrogate mother with two fostered golden pups derived from oviduct transfer of eight vitrified/thawed two-cell embryos. The pups were at the age of 10 days.

### Discussion

EG and DMSO have been widely used as permeating cryoprotective agents for embryo vitrification. EG is less toxic to embryos and is a weak glass forming molecule, whereas DMSO is a better glass forming molecule. The two are therefore often combined in vitrification solutions. It is quite often the case that solutions with 30% to 40% (or more) of permeating cryoprotectants are used for embryo vitrification. In this study, we compared different concentrations of cryoprotectants in vitrifying hamster embryos. Our results indicated that EDFS30 was more suitable than EDFS35 or EDFS40 (Table 1) for the vitrification of two-cell hamster embryos. Cryoprotectant solution containing 15% EG and 15% DMSO has also been successfully used in the OPS vitrification of mouse and goat embryos and in cryoloop vitrification of human embryos, but had not previously been used with hamster embryos. In the study reported by Lane et al., cryoprotectant solution containing 20% of EG and 20% of DMSO was used in both the cryoloop and OPS vitrification experiments. Our success in using lower concentration of cryoprotectants to vitrify hamster embryos may have provided a much improved cryopreservation protocol for hamster embryos – the lower concentration of cryoprotectants means less toxicity to the embryos.

Besides the concentration of cryoprotectants, exposure time in vitrification solutions affects the survival and subsequent development of vitrified embryos. When exposure time was prolonged to 120 s, the in vitro developmental ability of hamster embryos was reduced (Table 2). This is likely due to toxic injury caused by EG and DMSO. Therefore, to minimize toxic injury to embryos, we optimized the exposure conditions.
exposure time of embryos to cryoprotectant solutions. We found that 30 s and 60 s exposure times resulted in best blastocyst rates and that there was no difference ($P > 0.05$) in blastocyst rates between them. This finding indicates that exposing hamster embryos to cryoprotectant solutions for 30 s is enough to guarantee cryoprotectant permeation and to avoid intracellular ice formation; and this exposure time is comparable with the 25 s of exposure time reported in mouse$^{24}$ and goat embryos$^{41}$ during OPS vitrification. We also found that when 30 s of exposure time was used, there was no significant difference ($P > 0.05$) in blastocyst rates between embryos vitrified with and without pre-treatment. This finding is unexpected in light of what has been observed in other species. For example, Zhou et al.$^{24}$ have demonstrated higher developmental potential of mouse earlier stage embryos vitrified by the two-step method than by the one-step method; Han et al.$^{43}$ have also reported that survival rates and in vitro development of vitrified rat two-cell embryos are improved by the two-step method. The difference in species (hamsters vs. mice or rats), components of vitrification solutions, and embryonic cultural conditions may account for such a discrepancy. Even though similar blastocyst rates were observed between the two methods in our study, pretreatment for a short time with lower concentration of cryoprotectants may give embryos more time for permeation and dehydration, which may be beneficial in providing effectiveness and stability to OPS vitrification. Therefore, we chose the two-step method as the preferred protocol for OPS vitrification of two-cell hamster embryos.

Comparing the embryos vitrified at different developmental stages (from two-cell to morula), no significant difference ($P > 0.05$) in blastocyst rates (62–80%) was observed. Therefore, the present protocol developed for two-cell embryos may also be used for vitrifying hamster embryos at four-cell, eight-cell, and morula stages. However, we found that vitrification with EDFS30 had a detrimental effect on PN and blastocyst embryos – no vitrified PN embryos developed to the blastocyst stage and a lower proportion (24%) of blastocyst stage embryos recovered after cryopreservation. A previous study has shown that the permeability of embryo cell membranes increases further as the developmental stage proceeds.$^{44}$ It is possible that the optimum condition developed for two-cell embryos may not achieve sufficient permeation for PN stage embryos because of the relatively lower surface area of PN embryos. Insufficient permeation and subsequent ice formation may account for lower developmental ability. For the lower recovery rate of vitrified blastocysts, we suspect that injures to the embryos caused by ice formation in the fluid-filled blastocoel during cryopreservation could be an underlying cause.$^{45}$ To prevent this, longer exposure and more permeation should be used for PN and blastocyst vitrification, but toxicity of cryoprotectants has to be taken into consideration.

The significant reduction of the total cell number and slight increase of the ratio of ICM to total cells (Table 3) indicate a delayed development of vitrified two-cell embryos. Reduction of blastomeres has been reported in vitrified mouse,$^{46}$ bovine,$^{47}$ and human$^{48}$ embryos. However, Lane et al.$^{19}$ observed no effect of vitrification on the total cell number in blastocysts derived from vitrified two-cell hamster embryos. One of the possible reasons for the discrepancy in results is that the blastocysts for cell counting were collected at earlier stages by Lane et al., since the total cell number (19.6 ± 1.4, OPS vitrification)$^{19}$ is much lower than that (48.7 ± 3.3, Table 3) in our study.

When vitrified two-cell embryos were transferred to recipients, 29% of them developed to live pups, which was comparable to fresh embryos (with a rate of 40%). The results of this study validate our successful establishment of a reliable OPS vitrification protocol to cryopreserve two-cell hamster embryos. Since there were no significant differences ($P > 0.05$) in blastocyst rates observed between fresh and vitrified embryos at the two-cell, four-cell, or eight-cell stages, we further posit that vitrified four-cell or eight-cell embryos are suitable for embryo transfer.

Our conclusion is that we have established an effective two-step OPS vitrification protocol for golden Syrian hamster embryos. This technological advancement provides a reliable method of cryopreserving hamster embryos and should greatly facilitate the

### Table 4. In vivo development of two-cell hamster embryos vitrified by EDFS30 media.

<table>
<thead>
<tr>
<th>Embryos</th>
<th>No. of embryos transferred</th>
<th>No. of recipients with foster pups / No. of recipients [%]</th>
<th>No. of embryos transferred to recipients with foster pups</th>
<th>No. of live offspring</th>
</tr>
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<tr>
<td>Control</td>
<td>72</td>
<td>6/9 (67)</td>
<td>48</td>
<td>61</td>
</tr>
<tr>
<td>Vitrified</td>
<td>56</td>
<td>6/7 (86)</td>
<td>48</td>
<td>31</td>
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<td></td>
<td></td>
<td></td>
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<td>19 (40 ± 8)$^{a}$</td>
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<td></td>
<td></td>
<td></td>
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<td>14 (29 ± 8)$^{a}$</td>
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$^{a}$Values with same letters indicate there was no significant difference between two groups ($P > 0.05$).
development, propagation, distribution and application of genetically-engineered hamster lines.

Declaration of conflicting interests
Zhongde Wang is a co-founder of Auratus Bio, LLC, a biotechnology company specializing in creating genetically-modified animals for biomedical research and agricultural applications.

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References
Refinement of a thermal threshold probe to prevent burns

MJ Dixon¹, PM Taylor¹, LC Slingsby² and JC Murrell²

Abstract
Thermal threshold testing is commonly used for pain research. The stimulus may cause burning and merits prevention. Thermal probe modifications hypothesized to reduce burning were evaluated for practicality and effect. Studies were conducted on two humans and eight cats. Unmodified probe 0 was tested on two humans and promising modifications were also evaluated on cats. Probe 1 incorporated rapid cooling after threshold was reached: probe 1a used a Peltier system and probe 1b used water cooling. Probe 2 released skin contact immediately after threshold. Probe 3 (developed in the light of evidence of ‘hot spots’ in probe 0) incorporated reduced thermal mass and even heating across the skin contact area. Human skin was heated to 48°C (6°C above threshold) and the resulting burn was evaluated using area of injury and a simple descriptive scale (SDS). Probe 1a cooled the skin but required further heat dissipation, excessive power, was not ‘fail-safe’ and was inappropriate for animal mounting. Probe 1b caused less damage than no cooling (27±13 and 38±11 mm² respectively, P=0.0266; median SDS 1.5 and 4 respectively, P=0.0317) but was cumbersome. Probe 2 was unwieldy and was not evaluated further. Probe 3 produced even heating without blistering in humans. With probe 3 in cats, after opioid treatment, thermal threshold reached cut-out (55°C) on 24 occasions, exceeded 50°C in a further 32 tests and exceeded 48°C in the remainder. No skin damage was evident immediately after testing and mild hyperaemia in three cats at 2–3 days resolved rapidly. Probe 3 appeared to be suitable for thermal threshold testing.

Keywords
refinement, pain, nociceptive threshold, cat, thermal

Pain relief is an essential component of treatment for injury and disease; study of the applied pharmacology of analgesic drugs is required so that optimal treatments can be devised. It is particularly important that appropriate pain relief is provided for laboratory animals undergoing painful intervention. All analgesic drugs have side-effects and cannot be used indiscriminately. Those most appropriate for the prevailing conditions should be documented; and knowledge of their efficacy, safety and duration of effect can optimize pain relief in each species and circumstance.

Nociceptive threshold testing (NTT) is widely used in studies investigating pain and its treatment. This applies both when a laboratory species is used as a model for humans and when a potential analgesic is under investigation for therapy in the target species. Pain is a complex phenomenon involving both sensory and affective components, so investigation into its mechanisms requires that pain is inflicted during the study, and NTT commonly fulfils this requirement. Analgesic treatment generally increases NT,¹ thereby allowing assessment of the drug’s behaviour under controlled laboratory conditions without confounding from the inevitable variability encountered in clinical studies.

A variety of stimuli have been used for NTT. Of necessity they must cause pain, but, as described by Beecher,² the intensity of the stimulus should be related to the perceived pain intensity and should not result in

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lasting tissue damage or harm to the animal. The stimulus must also be repeatable, reliable, and easy to apply, and must result in a clear endpoint.

Thermal stimuli are commonly used in NTT. Such stimuli may, however, cause local damage, which if serious, makes the technique unacceptable. In some studies, attempts to reduce burn injury have led to the use of a low maximum ‘cut-out’ temperature if there is no response. Lowering the maximum temperature reduces the ability to detect an increased threshold, and may make the investigation worthless. It is essential that animals used in research are not wasted; any refinement which maximizes data robustness is worthwhile.

This paper reports refinements made to thermal NTT probes to allow sufficient ‘headroom’ above the baseline thermal threshold to reduce burning without compromising data collection. The original probes have been widely used in several species, and are well tolerated. However, the high thresholds resulting from treatment with potent analgesics have led to skin burns, even with the cut-out reduced to 55°C.

We hypothesized that reducing heat transfer to the skin should reduce burning and aimed to evaluate two methods of reducing skin damage: either by cooling the thermal probe after threshold is reached, or by decreasing the subsequent heat transfer by reducing probe skin contact. We hypothesize that either of these systems should reduce skin damage from prolonged contact between the probe and skin at threshold temperature (TT). A third probe design was also evaluated in the light of information collected during studies of the first two. The investigation was conducted after approval by the local institutional review board, and the investigations in cats were conducted under the Animals (Scientific Procedures) Act 1986. The human studies were conducted on two of the authors.

**Materials and methods**

**Original equipment**

A heater probe (probe 0) used for thermal NTT in cats was evaluated prior to and after a number of modifications. Probe 0 had been developed from the 9 x 8 x 5 mm probe (heating contact area 72 mm²) originally designed for thermal NTT in cats and used in numerous feline studies. (Figure 1). Probe 0 was smaller, and therefore had lower thermal mass than the original design. It contained the resistor heating element embedded in a minimal volume of thermally conductive epoxy with an adjacent temperature sensor, resulting in a 39 mm² skin heating contact area (Figure 2). Probe 0 weighed less than 5 g and heated at 0.6°C/s. The probe was connected to the control unit by a five-way ribbon cable, and incorporated a safety cut-out at 60°C which was subsequently reduced to 55°C. For thermal threshold testing in animals the probe was attached to a modified blood pressure bladder and held in place on the shaved thorax by an elasticated surcingle. The bladder was inflated manually to 100 mmHg to provide reproducible contact between the probe and the skin. A handheld switch activated the probe heater, and TT was recorded and held on the display when the animal reacted visibly to the stimulus. Simultaneously, the heater was switched off.

**Pilot studies**

Pilot studies were conducted on the forearms of two of the authors (MJD and PMT) using probe 0. For human testing the probe was mounted on a handheld...
pneumatic mechanical actuator (ProdPlus; Topcat Metrology Ltd, Ely, UK) in order to maintain constant contact with the skin. The heater was held against the hairless skin of the ventral antebrachium at 1.5N. Thermal nociceptive threshold (the first point at which the sensation was painful) was measured in both individuals as the mean of three tests taken at 5 min intervals at adjacent sites on the antebrachium. To examine each test probe’s characteristics heating was continued 6°C beyond threshold to a reading of 48°C and was then switched off. Skin damage was evaluated by visual inspection 20 h after all the tests.

Test probes

Three fundamental probe modifications were investigated. All systems were first evaluated on the bench to examine heating and cooling characteristics and assess potential for use in vivo. The internal probe sensor was used to track temperature and recorded using a data logger sampling at 10 Hz.

1) Cooling of the probe immediately after each test

Probe 1a. Rapid cooling of the probe was achieved by mounting a series of two Peltier heat pumps (9 x 9 x 3.4 mm, cat no 189–1576; 30 x 30 x 3.5 mm, cat no 693–7040; RS Components Ltd, Corby, UK) immediately behind the thermally conductive epoxy layer that surrounded the heating element. The pump was switched on at TT to conduct heat away to a heat sink. The heat sink, a 50 x 25 x 1 mm copper plate, was attached behind the Peltier pump.

Probe 1b. An alternative water-cooled probe was developed in which the heating element was placed inside a 9 mm long brass tube (ID 2.4 mm and OD 3.2 mm). A second brass tube of the same dimensions was mounted in parallel (soldered) and carried water from a 2 L reservoir containing ice and water mounted 60 cm above the assembly. Gravity-driven water flow from the reservoir was controlled by a roller constrictor (VYGON infusion set, model 027421; VYGON Vet, Swindon, UK) at 170 mL/min. Flow rate was calibrated by measuring the volume delivered over one minute. When heating was stopped, a tap was immediately opened manually allowing iced water to flow through the tube parallel to the heater and cool the probe (Figure 3).

2) Release of the probe pressure on the skin immediately after the endpoint

Probe 2. A vent valve (Bio-Chem part no P/N075T2NC12/32M; Biochem-Fluidics, Cambridge, UK) was incorporated into the air line supplying the pressure bladder holding the thermal probe against the skin. The valve was activated automatically when the heating was stopped at TT, thereby reducing probe–skin contact. Once the probe had cooled to room temperature, the bladder was re-inflated for the next test.

3) Reduced thermal mass with even heating

Probe 3. Probe 3 was developed in response to the pilot studies conducted on the authors’ forearms which suggested that heating from probe 0 was uneven (Figure 4). This was further supported by evidence of even heating in the non-cooled tests with probe 1b. For probe 3, the heating element was encased in an 8 mm long brass tube (ID 2.4 mm and OD 3.2 mm) to produce even heating without any hot spots (Figure 5). The thermal mass of the heating components was less than in probe 0. The brass tube was positioned behind low heat conducting plastic material with 0.4 mm depth exposed, giving a heating contact area of about 20 mm² when the plastic was held on the skin surface with the contour of the exposed brass tubing depressing the skin.

Human testing

Promising modifications were then tested on two of the authors (MJD and PMT) in order to compare the modified probe with the original (probe 0). Tests were performed on the hairless antebrachium and shaved medial lower tibia and each individual was blinded to the identity of the probe in use. The probe under test was held against the skin at each site at a constant force of 1.5N as in the pilot study. The probe was heated to 48°C (6°C above threshold). Each test was repeated three times with each of the original and modified probes in
random order. An adjacent site along the limb was used for each test. At least 10 min elapsed between tests on the same limb of either individual. The skin damage was described by a simple descriptive scale (SDS) scoring system (0–3, Table 1) and the area of skin damage was measured 20 h later (length and breadth by vernier).

Probe 1a. The Peltier pump-cooled probe, although effective on the bench, proved inappropriate and was not tested further.

Probe 1b. The water-cooled probe was tested in 24 randomized experiments (2 subjects, 2 limbs, 6 tests per limb): on each individual, on adjacent sites on both forearm and leg, three cooled to 9–10°C immediately after heating to 48°C, and three not cooled, where the probe was held on the skin without water flow for a further 2 min after heating was stopped.

This cooling method was considered to be potentially effective for large animals but was not tested further on animals in this investigation because, with the water reservoir and substantial tubing for sufficient water flow, it appeared too cumbersome for use in cats.

Probe 2. The pressure relieving method was also effective on the bench but was cumbersome and bulky, again unsuitable for mounting on an animal. Hence no further pressure experiments were conducted.

Probe 3. The low thermal mass probe was compared with the original in 24 randomized experiments as above: three at each site on each individual with each probe. Each probe was heated to 48°C (6°C above threshold) and the test subject was blinded to the identity of the probe. Skin damage was described by the SDS scoring system (Table 1) and the area was measured 20 h later.

Cats. Eight purpose-bred neutered adult domestic shorthair cats (2 male and 6 female) were studied. They were housed in groups of four in accordance with Home Office (UK) regulations. Testing was carried out on two cats at a time, in a separate room where they were housed individually within sight of each other in large cages. Cats were taken to this room the day before the study to become accustomed to the new environment, and were returned to their long-term housing after testing was complete. A minimum two weeks’ rest period was allowed between any repeat testing on the same animal.

Only the most promising probe modification was tested on animals. Hence the low thermal mass, uncooled tubular probe 3 (Figure 5) was studied in eight cats. The probe was used in a wireless threshold testing system operated by infrared control which

<table>
<thead>
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<th>Score</th>
<th>Description</th>
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<tbody>
<tr>
<td>0</td>
<td>No damage detected</td>
</tr>
<tr>
<td>1</td>
<td>Mild reddening or raised oedema &lt;2 mm depth</td>
</tr>
<tr>
<td>2</td>
<td>Marked reddening or raised oedema &gt;2 mm depth</td>
</tr>
<tr>
<td>3</td>
<td>Blistering and broken skin surface</td>
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Table 1. Numerical Rating Scale scoring system for skin damage.
allowed the animal free movement during testing and facilitated detection of a natural behavioural endpoint when TT was reached17 (WTT1, Topcat Metrology Ltd, Ely, UK).

The probe was placed on the shaved skin of the thorax as previously described11,12,18 and heated at 0.8°C/s until threshold was reached, detected by the cat’s response, generally turning to the site, jumping forward, twitching the skin or occasionally vocalizing. Three pretreatment tests were performed at 10 min intervals to confirm normal responses. Buprenorphine (20 μg/kg) or fentanyl (20 μg/kg) was then injected intravenously and threshold was measured eight times at 30 min intervals. The probe was not moved between tests and the skin was allowed to return to pretesting temperature without active cooling or removing the thoracic band. The skin was examined for any evidence of heat damage immediately, at 2, 15 and 24 h, and then daily for three days after the last test.

Historical data (unpublished) from four of the same eight cats, tested six months previously after similar opioid treatment using probe 0, were examined for descriptive comparison of skin damage to confirm published experience of the extent of post threshold testing skin burns in cats.

Data analysis. Human data from cooled and not cooled burn sites were compared using Student’s t-test for normally distributed data (area) and Mann–Whitney U-test for SDS. P < 0.05 was considered significant. No formal analysis was applied to the cat data.

Results

Pilot studies

Probe 0. Probe 0 produced blisters of 1–3 mm diameter at TT (42°C) indicating that the heater produced ‘hot spots’ where the skin reached temperatures considerably higher than those recorded by the sensor (Figure 4).

Human testing

Probe 1a. The heat was removed from the probe itself, but still needed dissipation from the site. This required a considerable bulk of equipment that was unsuitable for mounting on an animal. In addition, since Peltier heat exchangers are inefficient and consume a great deal of power, this proved too much for battery-driven equipment, further preventing the mounting of the system on the animal, or the use of light, flexible cables. A third disadvantage was that the system is ‘fail-dangerous’: if power is lost the extracted heat returns very quickly, leading to a rapid increase in temperature and a significant risk of serious burns. This was inappropriate for further testing.

Probe 1b. The ice water-cooled probe resulted in a significantly smaller area of damaged skin than when not cooled (27 ± 13 and 38 ± 11 mm² respectively, P = 0.0266) (Figure 6). The SDS scores were lower with the cooled probe than the not cooled (median 1.5 and 4 respectively, P = 0.0317).

Probe 2. Probe 2 proved too cumbersome for mounting on animals and was not evaluated further.

Probe 3. The low thermal mass/even heating probe (Figure 5) produced skin reddening after heating to 48°C over an area of the same shape and size as the probe footprint and with no blistering, indicating even heating across the sites with no evidence of ‘hot spots’.

Cats

Probe 3. In cats the mean ± SD skin temperature was 37 ± 0.4°C before testing. Mean ± SD pretreatment thermal threshold was 43.5 ± 2.0°C. Post opioid administration, all eight tests went to cut-out (55°C) in three cats and all eight tests were above 50°C in six cats. In the remaining two cats, one and four tests respectively exceeded 50°C, and the rest exceeded 48°C. Immediately after testing the imprint of the probe was evident, confirming the testing site, but there was no skin damage (Figure 7). There was no sign of damage at 2, 15 and 24 h, but three days after testing one cat had a mildly reddened area at the site of the brass tube heating element (Figure 8). Also at 24 h, two other cats had similar reddening in an oval shape that did not
clearly correspond with the probe site. These marks were entirely ignored by the individual cats and did not elicit any signs of pain or discomfort on palpation. They resolved completely within a few days.

Historical data using probe 0 in four of the eight cats recorded that, after opioid treatment, cut-out (55°C) was reached in 2/8 tests in only two cats. In one cat threshold never exceeded 44°C, and overall, 8/32 tests exceeded 50°C and a further seven exceeded 48°C. No skin damage was detected on the cat with thresholds <44°C, but 2–3 circular damaged areas were detected at the probe site in the other three cats. These resolved over the following few days and did not appear to cause discomfort.

**Discussion**

Thermal NTT has become a well accepted laboratory method of assessing the likely therapeutic efficacy of an analgesic. Studies in cats have also demonstrated that increased TT appears to correlate with clinical perception of the efficacy and duration of opioid analgesics. This method of analgesiometry is well validated and is now accepted for assessment of efficacy prior to analgesic drug registration. Early laboratory studies of buprenorphine in cats contributed data used in the successful application for buprenorphine UK Market Authorization (MA) for feline use in 2005 (Vetergesic, Alstoe Animal Health, York, UK) and this analgesic has had considerable impact on feline clinical pain management. Since evaluation of analgesics is a major application for NTT it is inevitable that the stimulus will frequently reach temperatures higher than the normal threshold and may burn the skin. Any improvements made to this methodology which reduce the heat transfer and subsequent injury has the potential for refinement affecting numerous laboratory animals.

We have demonstrated that adaptations can be made to the heating probe which reduce skin damage. This was effective under conditions occurring during laboratory testing of the most potent class of analgesics, the opioids, when TT may be well above normal for several hours.

The Peltier heat pump was effective in rapidly cooling the probe after heating. However it proved to be impractical for use with a probe designed to be attached to the subject. The process depends on a relatively inefficient use of power (http://en.wikipedia.org/wiki/Thermoelectric_cooling), and it proved to be impossible to run on batteries small enough to be mounted on the animal. In addition, removing the heat rapidly from the site requires a very large heat sink which itself needed cooling. All this was too bulky for small animal use. Commercially available devices which use
Peltier heat pumps to provide hot and cold stimuli such as the handheld Physitemp (http://www.physitemp.com) and the Medoc TSAII Neurosensory Analyser (http://www.medoc-web.com) incorporate a substantial probe connected to a large and bulky mains-powered control box via a thick, stiff cable which is required for power and water cooling. These are impractical for attachment to an animal and are unsuitable for use on unrestrained subjects. The final deciding feature which made this method unsuitable for further testing was its ‘fail-dangerous’ nature. It is essential that a stimulus probe fixed on the subject ‘fails-safe’ if power is lost for any reason. After a Peltier pump has withdrawn heat, and if power is lost the heat returns very rapidly, causing significant burning; which is clearly unacceptable for a remote-controlled probe attached to an animal.

A notable result from this investigation was confirmation that rapid cooling after threshold is reached is of considerable benefit in preventing skin injury. The iced water system was effective in cooling the skin and reducing heat injury; and although this is cumbersome for small animals such as cats and dogs, it has potential in larger species. Horses are often tested in stocks using equipment incorporating cables connected to bench top control units. Under these conditions the addition of an ice water reservoir and tubing would be quite feasible.

Heat transfer is influenced by probe pressure and its effect on skin contact. Reducing the contact between the probe and skin at threshold, so that no further heat is transferred to the skin should therefore reduce the transfer of remaining probe heat to the skin. Although the prototype system was effective in releasing the pressure at threshold, the valve and its connections were too heavy and bulky for use on a small animal such as the cat. Even with a larger animal the overall benefit of the additional complexity was questionable. However, this approach still merits further evaluation.

The tubular probe 3 allowed testing to threshold during opioid treatment in cats with virtually no subsequent skin damage. Although the burns encountered with the original 9 x 8 x 5 mm probe first described were not severe or extensive, and did not appear to cause discomfort, entirely preventing any burning would clearly be worthwhile. The heating contact area and the thermal mass of probe 0 were less than those of the original probe, but minor skin lesions were still reported. In probe 3 the thermal mass and heating contact area were further reduced. Reduction of the thermal mass in contact with the skin could be expected to reduce heat transfer to the skin; the maximum temperature reached is unchanged, but the probe cools faster after the stimulus is switched off. The value of reducing the thermal mass was not formally evaluated in the present investigation, but fundamental physical principles suggest that it should have some benefit. Burn injury relates to the amount of heat energy transferred to the skin. Thermal mass is an indication of the amount of heat the probe can store and is therefore available for transfer. Thermal mass depends on the size of the object and its specific heat capacity. Specific heat capacity is a property of the material, so a larger probe (of the same material) has greater thermal mass than a smaller one and therefore also provides more heat energy available for transfer.

It is likely that even heating across the whole contact area contributes substantially to preventing skin burns by eliminating ‘hot spots’ of higher temperature than the sensor’s readout. Although the temperature reached at the hot spots was not precisely known, it must have been higher than the displayed temperature as less than one minute at 42°C is very unlikely to cause damage. The lesions seen with probe 0 both on human skin (Figure 4) and in cats used in previous studies were small spots in the centre of the probe contact area where the maximum heat is generated by the resistor. Although embedding the sensor and resistor in thermally conductive epoxy facilitates heat transfer, the temperature across the probe surface is still not uniform. Skin in contact with the centre of the resistor will reach a higher temperature than the sensor separated from the resistor by epoxy. Placing the resistor inside brass tubing ensures consistent heat transfer to both skin and sensor, as thermal conductivity of brass is much greater than the epoxy. Hence the temperature across the probe 3 contact area was consistent and was more accurately recorded by the sensor. A recent report of thermal NTT in cats may support this view: two cats treated with tramadol and morphine developed minor skin lesions a few days after testing even though the peak mean threshold was only 45°C. It is plausible that the probe may have produced hotter areas than detected by the sensor. The historical data from probe 0 presented here may be an additional reflection of this. Most of the cats experienced some burns with probe 0, in spite of fewer post opioid tests reaching cut-out (55°C) than with probe 3. This may be a result of small areas of skin reaching temperatures higher than those detected by the sensor, and triggering a response. In addition, the baseline feline threshold recorded with probe 3 (43.5 ± 2.0°C) was higher than that in older published studies using probe 0 or earlier designs (40–42°C). These observations support the concept that uneven heating leads to ‘hot spots’ above the displayed temperature. This may not only cause burns but may also lead to TT recorded at lower values than reality.
A higher heating rate was used with probe 3 than that employed previously with both the original probe and probe 0. The effect of heating rate on burning was not examined in this investigation. From fundamental principles, as the area under the temperature–time curve (AUC) is smaller, a higher heating rate is more likely to decrease heat transfer and the likelihood of burning. This suggests that the AUC should be kept as low as possible. Even relatively low temperatures (44°C) for a long period of time (5 h) caused burns, a slower rise in temperature will tend to increase the risk of burning as the AUC is larger. This is difficult to study on the bench as the effective heating rate can only be measured when the probe is heated in situ on the subject: heat transfer from the probe and hence its actual temperature during heating are affected by the thermal conductivity of its surroundings. Heating rate is faster in air than on tissue, as air is a relatively effective insulator. Various adjustments in heating rate and probe design have been made in previous investigations, particularly when adapting the system to new species; horses appeared to burn easily. Reducing the heating rate appeared to reduce skin injury in horses, but this was already minimal in the study reported. It is also recognized that fast nociceptive stimulus ramps lead to higher thresholds primarily because the stimulus overshoots further after the control is switched off. The effect of heating rate on tissue injury merits further investigation.

The fundamental approaches to reducing skin damage were first evaluated on ourselves, thereby including subjective sensation as well as avoiding unnecessary use of animals. We studied cats to test the most promising probe design as there is a considerable body of information from feline thermal NTT. Cats are generally regarded as a difficult species for NTT, due to their size and temperament, so we hypothesize that equipment they tolerated is likely also to be suitable for other species. However this approach prevented further evaluation of the water-cooled system.

In conclusion, the low mass of feline tissue, even-heating probe appears to offer the best features of those evaluated, being the most practical for mounting on an unrestrained small animal. Water cooling was effective in cooling the probe and reducing the potential for skin injury, but is only suitable for larger animals. The even-heating non-cooled design is now used in thermal threshold testing equipment supplied by Topcat Metrology Ltd.

**Funding**

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**Conflict of interest statement**

PM Taylor and MJ Dixon are directors of Topcat Metrology Ltd.

**References**

The effect of anaesthesia on somatosensory evoked potential measurement in a rat model

Jukka Kortelainen¹,², Hasan Al-Nashash²,³, Ashwati Vipin², Xin Yuan Thow² and Angelo All⁴,⁵

Abstract
Somatosensory evoked potentials (SEPs) are widely used to study the functional integrity of ascending sensory pathways. For animal studies, SEPs provide a convenient method to quantitatively assess the functionality of the nervous system with low invasiveness. Even though they are frequently used in animal models, little attention is paid to the fact that SEPs are vulnerable to contamination from experimental factors such as anaesthetic delivery. In this study, the effect of isoflurane on SEP measurement was investigated in a rat model. The aim was to find out the adjustments for anaesthetic delivery optimizing the quality of the recordings. Two aspects were studied: the effect of isoflurane dosage on the SEP parameters and on the repeatability of the measurements. The SEP quality was found to be best when 1.5% isoflurane concentration was used. This dosage resulted in the best signal-to-noise ratio and equal repeatability of the measurements compared with the others. Our findings can help in refining the anaesthetic protocols related to SEP recordings in a rat model and, by improving the quality of the measurements, potentially reducing the number of subjects needed to carry out studies.

Keywords
anaesthesia, animal model, reduction, rodents

Somatosensory evoked potentials (SEPs) are widely used to study the functional integrity of ascending sensory pathways. In current clinical practice, they are used for various diagnostic purposes such as intraoperative monitoring during high-risk surgery and the detection of hypoxic ischaemic encephalopathy after cardiac arrest.¹,² For animal studies, SEPs provide a convenient method to quantitatively assess the functionality of various portions of the somatosensory pathways with low invasiveness. Even though several sophisticated measures for the quantification of SEPs have been proposed,³,⁴ the analysis still mainly relies on the determination of simple parameters such as amplitudes and latencies from the averaged signal waveform.⁵ Whereas these parameters are rather easy to derive from the recordings, they are also vulnerable to contamination from experimental factors present during data collection. This artefact, increasing the variability of the data, may lead to the use of an unnecessary high number of subjects in the study when, for example, statistical significance is of interest.

Anaesthetics are known to influence neural electrophysiology.⁶–⁸ In animal studies, maintaining a

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steady-state anaesthesia with inhalational agents such as isoflurane is more suitable compared with drugs administered by, for example, injection. However, these gases are known to affect SEP features such as the amplitude of the signal, and thus anaesthesia has a critical influence on the recordings. To this day, no standardized protocol or justifiable recommendation exists for the anaesthetic delivery which guarantees the quality of SEP recordings in the rat model.

In this study, the effect of isoflurane on SEP measurement was investigated in rats. The aim was to find out the appropriate adjustments for anaesthetic delivery to optimize the quality of the recordings. Two aspects were studied: the effects of isoflurane dosage on the SEP parameters and on the repeatability of the measurements. The study was approved by the Institutional Animal Care and Use Committee (IACUC) at the National University of Singapore, Singapore.

To carry out SEP measurements, four screw electrodes were implanted on the cranium of 10 adult female Sprague–Dawley (200–220 g) rats. Rats were ordered through ACUC office at the National University of Singapore and were supplied by the “InVivo Inc.” in Singapore. The electrodes were located above the somatosensory cortex of different limbs according to Figure 1. A fifth electrode was positioned in the right parietal area close to the lambda to serve as a reference. Carboxylate dental cement (3M, St Paul, MN, USA) was applied in order to fix the electrodes to the cranium. During the electrode placement, the animals were anaesthetized with a ketamine (75 mg/kg) and xylazine (10 mg/kg) cocktail.

The SEP recordings were carried out as illustrated in Figure 1 at least seven days after the electrode implantation (see Supplement 1 online at http://jansagepub.com for details). During the measurement, the rats were anaesthetized with a mixture of isoflurane and 100% oxygen delivered at a rate of 1.3 L/min. The anaesthesia was maintained using a rodent-size anaesthesia mask connected to a diaphragm with a C-Pram circuit (Smiths Medical, Dublin, Ohio, USA) designed to deliver and evacuate the gas. The recordings were made while the isoflurane dosage was increased in a step-like manner from 1.0% to 2.5% using 0.5% increments. At each step, the dosage was kept fixed for 5 min to guarantee the equilibrium before recording the SEPs. To study the effect of the anaesthetic dosage on the repeatability of the measurements, another SEP recording was carried out for each rat at least one day after the first one following the same protocol.

From the SEP waveforms, two parameters were defined: amplitude representing the total peak-to-peak value of the signal within 5–40 ms after the stimulation and N1 latency representing the time from the stimulation to the first negative peak in the signal. The repeatability of the measurement was assessed by calculating the difference in the parameter values between the first and second recordings for each rat. For the analysis, only the hindlimb recordings were used.

The effect of the isoflurane dosage on the SEP waveform, derived parameters, and repeatability of the measurement is illustrated in Figure 2. Increasing the concentration from 1.5% to 2.0% and finally to 2.5% resulted in a statistically significant decrease in the SEP amplitude. Furthermore, a slight increase in the N1 latency was observed which was, however, not statistically significant. Furthermore, the anaesthetic concentration did not have a statistically significant effect on the repeatability of the measurement, evaluated by the difference in the parameter values between the first and second recordings from the rats, suggesting comparable repeatability at all anaesthetic levels. The recordings could not be carried out at 1.0% isoflurane as the rats tended to have some unintentional limb movement at that concentration.

Even though SEPs are often used for quantitative analysis of neural function in animal models in the literature, little attention is paid to the fact that the measures are vulnerable to contamination from experimental factors such as anaesthetic delivery. Neglecting this source of artefact may lead to increased variability.

Figure 1. Block diagram of the experimental system and electrode locations for somatosensory-evoked potential (SEP) measurement. The forelimb recording sites [stars] are located 0.2 mm posterior and 3.8 mm lateral to the bregma. The hindlimb recording sites [circles] are located 2.5 mm posterior and 2.8 mm lateral to the bregma. Reference electrode [square] is located 3 mm lateral to lambda.
in the data and consequently, for example, raise the number of subjects included in the study unnecessarily. The current study showed the crucial role of a correct anaesthetic dosage in carrying out accurate SEP measurements using rats. In line with previous findings, increasing the anaesthetic concentration significantly suppressed the SEP amplitude, emphasizing the importance of carefully controlled fixed anaesthetic delivery during the measurement and between consecutive recordings. Based on the results of the current study, 1.5% isoflurane concentration is suggested to be used when SEPs are being recorded in a rat model. This dosage resulted in the highest SEP amplitude and thus the best signal-to-noise ratio which is of interest especially when dealing with a model which includes possible neural injury. Lighter concentrations led to inadequate anaesthesia and unintentional limb movement which contaminated the recordings. A 1.5% concentration achieved the best signal-to-noise ratio; and equally, or even more, important to this was the fact that the repeatability of the measurements was comparable with that of the other concentrations. This indicates the stability of the recordings at this lowest feasible concentration which, in combination with this optimal signal-to-noise ratio, guarantees the best conditions for SEP measurements. Even though in this study only the effects of isoflurane were being studied, similar results can be expected when using other anaesthetics with GABAergic pharmacodynamics. With these drugs, a similar approach is suggested when optimizing the anaesthetic delivery to achieve a high quality in SEP recordings.

Declaration of conflicting interests
None declared.

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References


Neotropical primate nursery in a squirrel monkey breeding unit in Brazil

BCS Meireles, B Goldschmidt, FC Resende, CAA Lopes and LWF Nascimento

Abstract

Saimiri (squirrel monkey) is a neotropical primate of the Simian genus that has been bred in captivity for the development of research into human and animal health. They have been widely used in studies in ophthalmology, toxicology, pharmacology, psychiatry, neuroscience, vaccines and drug tests (such as malaria and measles agents), as well as effects on interactive behavior and cognition of Creutzfeldt-Jakob disease in man. The main concern of non-human primate (NHP) research centers is focused on the establishment of self-sustaining breeding colonies providing good quality research animals. Maternal rejection, dystocia and pneumonia are the main causes of newborn deaths in these species. Therefore, in order to ensure the survival of these valuable animals, the Laboratory Animals Breeding Center of the Oswaldo Cruz Foundation (CECAL)/Fiocruz, Rio de Janeiro, Brazil, has developed a protocol for the nursery rearing of these infants.

Keywords
squirrel monkeys, nursery, breeding, neonates, hand-rearing

Non-human primates (NHPs) represent valuable models for human disease research, since they are phylogenetically close to humans, sharing physiological, anatomical and genetic similarities.1

It is difficult to construct an overview of the use of NHPs in research, since several countries do not provide this information. However, a survey estimating the annual use of NHPs in research suggests a number of around 100,000 to 200,000, with 67% of studies involving Old World Monkey (OWM) and 15.5% New World Monkey (NWM) primates.2

The Saimiri genus is one of the most commonly chosen among the New World primates and has been used in the development of vaccines against malaria and measles, with a focus on ophthalmology and color blindness research. The study of malaria is of great importance since it affects one-quarter of the world’s population, killing about three million people every year. Its severity is such that the World Health Organization (WHO) considers malaria to be a major public health problem in many countries, particularly developing countries.

The large demand for these primates in biomedical research has led institutions to invest in their captive breeding, focusing on the reproduction and management of animals with a known origin. The use of purpose-bred animals of known origin reduces the number of animals needed and provides better research animals for investigators.

Of the five species of the genus, two are considered to be vulnerable (Saimiri oerstedii and Saimiri vanzolinii) and one is near threatened (Saimiri ustus). Saimiri sciureus and Saimiri boliviensis are not threatened; however, like most species of NHPs, the free-living populations are in decline.3

The females begin their reproductive periods at approximately 36 months of age and their estrous cycle lasts from 7–12 days. The gestation period ranges from 165–172 days.4 The estrous cycle in Saimiri spp. varies according to colony conditions, such as temperature, humidity, and the social organization of the group. Most births take place between 02:00 h and 06:00 h.5 Although widely used in research, little is known about the reproductive physiology of

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Saimiri spp. Although providing a reasonable rate of conception in captivity, they are very susceptible to stress, with high abortion and neonatal death rates, as well as infant rejection by their mothers. Theft of infants by non-lactating females in the same family group also occurs, which can lead to the deaths of the newborn. Usually the female gives birth to a single offspring which is carried on the back of its mother until weaning at six months old. Newborns weighing less than 90 g may be considered to be premature. Those weighing less than 80 g rarely survive. In healthy animals, birth weight ranges from 98 to 130 g, which is significantly higher than weights typically recorded for wild animals, probably due to extra nutritional care. The newborn weight is about 20% of the maternal body weight. Infants are predisposed to hypothermia probably due to their small size, large body surface area, and minimal body fat. The normal temperature for neonate squirrel monkeys ranges from 35 to 37°C, with lower degrees in their extremities like fingers and toes. The normal temperature for adults ranges from 38 to 39.5°C.

Dystocia and trauma account for 53% of neonatal deaths. Of these, 60% occur before the second day of life. Infections such as pneumonia, enteritis, meningitis, and septicemia cause more deaths in infants aged over two days. Infections are causes of death in 14% of infants which die before the second day of life. Infections followed by death also affect 30% of infants between two and seven days and 70% of infants between eight and 30 days. Cases of deaths that occur during the first 24 h of life are considered to be of intrauterine origin. In a study conducted with 51 births of squirrel monkeys, 15 neonatal deaths occurred, five as a result of skull fractures.

Immune response of hand-reared animals may differ from those reared with their mothers in two ways: a lack of mother’s milk, and an absence of physiological regulation from maternal contact. Mother’s milk is important because it contains growth factors, immunoglobulins, cytokines, and antimicrobial agents, and is nutritious. Maternal contact provides warmth and tactile stimulation which allow the infant to devote more energy for growth and maintenance instead of thermoregulation. The practice of rearing infants in a nursery may prevent female infants from learning maternal skills, which causes the rejection cycle to continue.

Providing proper environmental enrichment during the development of hand-reared infants is fundamental to the quality of life and welfare of the animals since they are raised without their mothers. These enrichments should help them develop sensory and tactile skills, and perception which enable the development of self-confidence and social interaction with other group members.

This study aims to describe a protocol for management of hand-reared neonate Saimiri spp. at the Laboratory Animals Breeding Center (Fiocruz, Rio de Janeiro, Brazil) in order to ensure the survival of those animals which are orphaned or rejected by their mothers.

Materials and methods

The Laboratory Animals Breeding Center at Fiocruz has around 231 S. sciureus and 14 S. ustus totaling 245 animals. Both of these species bred at the Fiocruz Primate Center came from a scientific rescue in the Amazon region in 1987. A second group of S. sciureus was donated by the Pasteur Institute of Cayenne in French Guiana in 2009. There are two types of enclosures for Saimiri spp.: the neotropical pavilion (NP) and the neotropical cage (NC). The construction of an NP includes a concrete structure covered by fiberglass tiles. The sides are made of steel screen and glass which allow the use of natural light, temperature and humidity, which approximates the laboratory environment to the natural habitat of this species. The cages are arranged in double lines forming two sets of seven cages each, with a circulation corridor between them. Each cage has an area of 7 m² (2 m width × 3.5 m depth). The animals are housed in groups of 8–12 animals per cage, consisting of two males, about five females and their respective offspring, varying from one to five infants. Each NC consists of a complex of six modules built in brick and iron grilles with anticorrosive treatment located in a partially wooded area. Each module has two cages with an outdoor area of 17.10 m² and an inner (refuge) area of 2.85 m², making a total of 19.95 m², with a height of 2.80 m and a common service corridor. In the NC the animals are housed in groups of 18–20 animals per cage, consisting of two or three males, about ten females and their respective offspring, varying from six to ten.

Due to the Brazilian climate, a protection system against extreme cold is not necessary. In Rio de Janeiro (Fiocruz area) the average annual temperature is 23.8°C and humidity ranges from 77% to 80%. The lighting period consists of 12 h of light in the summer and 11 h of light in the winter. Most enclosures are outdoor facilities, where temperature and humidity vary according to the daily climatic conditions. However, all enclosures are equipped with sprinkler systems that provide cooling on days when temperatures are above 30°C. Polypropylene nests are used to protect infants on cold days.

The animal diet is composed of two types of feed, ‘dry’ and ‘wet’. The dry diet formula is a pelleted feed (Nuvilab Primatas P®, Nuvital Nutrientes Ltd.,
Curitiba, Paraná, Brazil) which is made up of protein 24%, fat (ether extract) 9%, carbohydrate (nitrogen-free extract) 41%, fiber 5%; dry matter 88%; ME (kcal/kg) 3585.02; Ca 1.5%; P 0.8%; ash 9%. The food is supplied at fixed and permanent times. The first hours of daylight are the preferred time of feeding of the dry food (pellet diet) as these are diurnal animals. Food not consumed within 24 h is removed and replaced with a new portion. The fresh foods, such as banana, apple, mango, carrot, orange, acerola fruit, watermelon and eggplant are sanitized by immersion in chlorininated solution (50 ppm or 50 mg/L) for 15 min without rinsing, and are offered routinely and at one time during the early afternoons. Quail eggs are also offered twice a week, and insect larvae (*Tenebrio molitor*) once a week.

In Brazil, births of *Saimiri* spp. occur in the spring or summer, usually between October and February. When there is a birth, the conditions of the neonate and its mother, maternal care and risk of theft by another female are monitored. If any change that puts the neonate’s life at risk is detected, the mother and its infant are removed for clinical exams and sexing.

When a newborn squirrel monkey is found to be off its dam, and all attempts to reunite the infant to its mother or foster parent fail, it is taken to the nursery. In cases of hypothermia, dehydration, malnutrition, absence of reflexes to hold to the mother, or in cases of serious injury, the newborn is taken to the clinic. Clinical examination is performed with an assessment of the oral cavity to make sure that the airways are free, heart and lung auscultation, and measurements of rectal temperature and blood glucose. Evaluating for possible fractures, abdominal palpation and skull examination to assess any presence of trauma are carried out. Monitoring of seizures, and neurological reflexes like eyelid and plantar flexion are undertaken. The level of dehydration, and presence of diarrhea or vomiting are also checked. During treatment the aim is to stabilize the animal, often with oxygen and oral glucose administration, as well as to keep it warm with a thermal mattress or incubator. Depending on the newborn and the mother’s condition after stabilization, the caretakers will then decide whether to return it to the original enclosure group or place it into nursery care.

The team formed to take care of the infants consists of one veterinarian and two laboratory animal technicians who do not work with any other animals of the colony, thereby preventing cross contamination among the different simian species. Personal protective equipment (hats, masks, face protection, gloves, aprons, disposable shoes) should be used when handling animals, manipulating food and washing cages. Employees who work directly with the infants should be up to date with their vaccinations and vermifugation, and should present no symptoms of any infectious disease (such as influenza, herpes or conjunctivitis).

At Fiocruz, the nursery is installed in a room dedicated to infant care. Air temperature is maintained at an average of 26°C using an air-conditioning system. Relative humidity is maintained between 60 and 80%. Light–dark cycle is regulated with an artificial 12:12 h light–dark regime. There are individual cages with thermal mattresses. A cloth foster mother is provided to orphans, to which the neonates can cling. Items such as toys and environmental enrichment equipment (Figure 1a) are also offered. Once the infant comes to the nursery, the first feed is given as a 5% dextrose solution in 1 mL disposable syringes without a needle, to enable it to get used to being fed by hand and to reduce the danger of aspiration and pneumonia. The following feed is performed with a 1:1 ratio mixture of 5% dextrose and commercial human milk formula (NAN Comfor 1®, Nestlé Brasil Ltd., São Paulo, SP, Brazil). Subsequent feeds are provided only with this commercial milk, which is made up of protein (70% whey, 30% casein protein); fat (97% vegetable fat palm oil, palm kernel oil, canola oil, corn oil, soy lecithin and 3% milk fat from cow’s milk protein); carbohydrates (100% lactose) and prebiotics (4 g/L 90% galacto-oligosaccharides and 10% fructo-oligosaccharides).

An individual record is opened with the complete birth history, parents’ identification, sex, weight, and nursery entry day. Besides this information, a control table is compiled of milk ingested by the newborn, with the amount of milk in mL/h, and a space for notes such as medications or any other important data that may need to be recorded. The infant should be weighed and its temperature measured daily.

Neonates are placed in incubators or individual cages, and when possible in groups, because of the need of social interaction among NHPs. If there is a sick animal, this should be handled last to avoid any contamination of healthy infants. A technician is also assigned every night since neonates also need to be fed during this period.

Newborns are cleaned using a gauze soaked in warm water, taking special care with their genitals. They are then dried with sterile dry gauze. After the first month of life the infants are allowed to sunbathe for 20 min with a gradual increase of the exposure period of up to one hour, always in the early morning (between 08:00 and 09:00 h). Observations and activities during the clinic and health examination include: assessing stool consistency, abdominal distension by gas, activity or prostration, degree of hydration, fur appearance, mucosal coloration, rectal temperature, weighing, cleaning conditions of the bedding and milk bottles. Nystatin ointments + zinc oxide (Medley™ indústria
farmaceutica Ltd., Campinas, SP, Brazil) are used in infants with diaper rash. They receive the human NAN Confor\textsuperscript{1} milk powder in a proportion of 4.3 g to 30 mL of filtered water. The newborn Saimiri usually accept 1–2 mL of milk which is offered from a 1 mL disposable syringe without the needle once per hour. The amount of milk increases gradually with the animal’s development (Table 1).

During lactation, the animals are placed in a vertical position to prevent choking from the liquid feed (Figure 1b). Over time, the animal begins to feed itself from a milk bottle attached to the cage (Figure 1c). Some animals do not accept the milk well. In such cases, there are a few options to attempt: (1) to dilute the milk further (one measure of NAN\textsuperscript{TM} milk to 40 mL of filtered water), or (2) to sweeten the milk lightly. After use, the

*Figure 1.* (a) Young Saimiri sciureus on a hammock. (b) Twenty-two-day-old Saimiri sciureus stuck to a plush toy and being breastfed. (c) Animal feeding from bottle fixed to the cage.

*Table 1.* Feeding before weaning.

<table>
<thead>
<tr>
<th>Age</th>
<th>Frequency</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–1 month</td>
<td>Every 1 h from 08:00 to 22:00 h. Between 22:00 and 08:00 h feeding should be every 2 h.</td>
<td>Week 1: 0.5–2 mL milk&lt;br&gt;Week 2: 2–3 mL milk&lt;br&gt;Week 3: 3–6 mL milk&lt;br&gt;Week 4: 6–10 mL milk</td>
</tr>
<tr>
<td>2 months</td>
<td>Every 2 h from 08:00 to 22:00 h. Between 22:00 and 08:00 h feeding must be provided every 3 h.</td>
<td>10–15 mL milk</td>
</tr>
<tr>
<td>3–4 months</td>
<td>Bottle containing milk and another with water attached to the grid cage. Fruit and crushed ration offering begin.</td>
<td>30 mL bottles are reset several times a day, according to milk intake.</td>
</tr>
<tr>
<td>5 months</td>
<td>Fruit and ration in pellets are provided during the course of the day. First 2 weeks: three bottles at intervals of 4 h. 3rd week: two bottles with an interval of 8 h. 4th week: only one bottle in the morning.</td>
<td>Water, fruit and ration in pellets offered at ease.</td>
</tr>
<tr>
<td>6 months</td>
<td>Weaning</td>
<td>–</td>
</tr>
</tbody>
</table>
bottles are cleaned with water and mild detergent and sterilized for 10 min by boiling.

After weaning, a process that takes about six months, the animal is returned to its family group, along with other adults. This process is both gradual and slow. During the first week the animal is placed in a transport cage with food and water, in front of its home cage. After this time, the transport cage is placed inside the enclosure for interaction with other animals of the group. At night, the animal returns to the nursery unit. If after the first week there has been no aggression, and the rest of the group show acceptance of the infant, the transport cage is opened. If adaptation fails, the same procedure will be carried out with another group: this animal is removed at night and sent to the nursery. If the process takes place without problems and the infant is accepted well by the group, then the adaptation is considered to be satisfactory.

The breeding colony was maintained in compliance with Brazilian law, registered in IBAMA (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis) under the protocol number 77933 and approved by the Ethics Commission on Animal Experimentation of the Oswaldo Cruz Foundation under protocol number LW-24/09.

**Results and discussion**

A survey of infant NHPs of the *Saimiri* genus submitted to nursery-rearing at the Fiocruz Primate Center was performed from 2007 to 2014.

The reasons that led to the breeding of the *Saimiri* spp. infants in nursery were: mother’s death, maternal rejection, hypothermia and hypoglycemia due to lack of breastfeeding. Females with low maternal ability induce death of their young by abandonment.

Most infants sent to the Fiocruz nursery were one week old or less, as this phase is the most critical for survival. Of the 244 squirrel monkeys born in this center from 2007 to 2014, 156 were alive (63.94%), of which 13 (8.33%) were sent to the nursery where eight (61.54%) were brought up successfully, being re-introduced into the colony. Among opportunistic infections are bacterial agents like *Escherichia coli* that may result in newborn death and *Candida* spp. in animals undergoing antibiotic therapy. Infection by *Herpesvirus saimiri type 1* is relatively common and may develop ulcers that make it difficult to eat. Treatment is limited to supportive care, fluids, electrolyte balance and intake of caloric foods. The most common cause of death in nurseries is pneumonia secondary to milk aspiration. In agreement with these data, at the Fiocruz colony, the most common causes of infant death have been hypothermia, hypoglycemia, and pneumonia which usually lead to death in 66.66% of newborn monkeys within 48 h of life. Underweight animals are less likely to survive. It was observed that infants weighing less than 95 g were unable to survive. In our study, the minimum weight for successful survival was 103 g. The average weight taken of eight hand-reared animals at 11 months of age was 565 g, while the average weight of eight animals raised by their mothers at the same age was 545 g, demonstrating an advantage to physical development when raised in the nursery.

*Saimiri* spp. requires a diet of high calorie densities. The milk produced by *S. boliviensis* was analysed and showed a mean of 4.56% fat, 3.59% crude protein, 6.98% sugar, 16.59% dry matter and 0.91 kcal/g, which is similar to human milk and can be substituted with the commercial milk NAN Confor 1. Studies have shown the effects of omega-3 deficiency from the visual development in human infants and monkeys. NAN Confor 1 milk contains omega-3 in its composition, and no evidence of visual deficiency has been observed in animals reared in the nursery at Fiocruz.

During the first month of life, the infants are very quiet, no major movements are observed and they always cling to the plush mother. After this period, they begin exploring their cage and climbing the railings. This display of independence and environment exploration is intensified until weaning. Small breeding groups of infants of approximately the same age were formed to stimulate socialization. This social interaction in the cage can also serve as an overcoming response to separation from their mothers.

Weaning in nature occurs at around six months of age and it was observed that 6-month-old infants reared in the nursery were able to live with other members of the colony, including the adults. Re-introduction of the hand-reared infant was possible once the infant was capable of self-feeding. Despite the high costs involved, hand rearing infants in the nursery is still worthwhile, owing to the high probability of success and the value of animals. It is also in keeping with the principle of the 3Rs (reduction, replacement and refinement).

There is no evidence that animals reared in a nursery are more susceptible to infections in adulthood, and no stereotypes have been reported. Animals reared at the Fiocruz nursery have been well accepted in the social group and reproduce normally in captivity, with no history of behavioral or reproductive problems.

Therefore, the 61.54% success rate for *Saimiri* spp. bred in the nursery is satisfactory when compared with the 62% rate for *S. sciureus* bred by their mothers. Although the cost of maintaining these animals in the nursery is high, the possibility of preserving their lives
represents an invaluable benefit given the high value of these models.

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References
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Report from the SGV meeting

Background information

Recent reports and developments on sex bias in basic and preclinical research prompted the organization of this symposium. Different points were addressed by different specialists and are briefly summarized below.

PD Dr Birgit Ledermann, President SGV, Basel, Switzerland: Welcome introduction of the two day meeting

Summary of oral presentations

Dr Marcel Gyger, Lausanne, Switzerland: Form A point 51.3: Word of the VAUD Animal Experimental Commission President

It is often said that the estrous cycle of female mice leads to greater variability in data than in males. The recent papers of Zucker et al., have challenged this view quite drastically; males can be as variable than females. This raises the question of whether sex of the animals used in the experiments should be appraised more clearly in Form A by the local ethical review board? Form A is a mandatory form to be filed for seeking permission to perform animal experimentation in Switzerland. A qualitative assessment of several researchers’ answers to the question of which sex they selected in their research was done. Concerns of researchers were of three kinds besides the fact that some vital processes or diseases are clearly sex-linked. The first concern was not to increase the number of animals by using additional females for 3Rs reasons. Second, females are preferred because they are less aggressive than males, and third often researchers do not have the necessary human and financial resources to include both genders in their studies. It is obvious that many studies include only one sex and that this choice is often not scientifically relevant. This sex-biased research should be reevaluated and measures should be implemented to have a sex-balanced approach in research.

Professor Irving Zucker, University of California, Berkley, USA: Sex bias in animal studies

Animal models in the early part of the 20th century relied on cats and dogs. An increase in the use of rats was observed in the 1950s, a peak in the use of non-human primates in the 1980s, and a sharp increase in the use of mice in 1989 that was related to transgenic technology. The rise in the use of males in experimentation in the 1960s is explained by the fact that studies prior to the 1950s, where no sex was indicated, were mostly done on males.1 There is a long list of sex differences, running into the hundreds – for example, anxiety, pain, aggressive behavior, anesthesia, neurotransmitters, and brain structure. But are researchers aware of such differences? A meta-analysis included 1600 articles from 42 journals in 2009.2 Most research in many biological disciplines is conducted primarily on males. Sex bias is most pronounced in neuroscience and pharmacology. Several recommendations are given to avoid sex bias, both sexes should be studied in sufficient numbers. Funding agencies should take actions to favor studies on females.

Professor Neil Bradbury, University of Chicago, Illinois, USA: Do you know the sex of your cells

Why should a researcher care about the sex of the cells under investigation unless they work with specific sex-dependent diseases? The American Journal of Physiology indicates that species, sex, strain and race should be stated, whereas 75% of papers published in this journal do not state the sex of the cells. Genetic differences are intrinsic and related to sex chromosomes (5% of the genome is encoded by sex chromosomes), i.e. the zinc finger genes ZFY and ZFY. X-linked diseases are more prevalent in males, while hemizygosity in males can be lethal. There is a selective X-inactivation in females, but not all genes are subject to X-inactivation. The sex of mammary and prostate cells is

The Schweizerische Gesellschaft für Versuchstierkunde (SGV) meeting was held on 24 and 25 November 2015 at the University Hospital (CHUV) in Lausanne, Switzerland. On the first day the topic dealt with an important and hot issue: Why sex is important in research (this symposium was supported by Laboratory Animals Ltd, the program of the meeting is available from http://www.sciencesnaturelles.ch/organisations/sgv/meetings). Attendance at the meeting counts towards continued education days, and continued education is mandatory for all researchers who handle animals. The SGV sessions were well attended with up to 400 participants per day.

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obvious. Cell line sex determination can be used to identify X or Y genotype, based on a six base pair insertion in ZFY, by the presence of two or a single peak for XY or XX genotype, while variability indicates XXY or XYY karyotypes. Unfortunately, in many transformed cell lines the Y chromosome gets degraded and presents an XO karyotype. Moving towards personalized medicine it becomes important to mention the sex of animals and also that of cell lines when reporting biomedical data.

Professor Eleanor N Fish, University of Toronto, Toronto, Canada: SeXX matters in immunity

Sex influences multiple aspects of the immune-phenotype. Sex effects on genes are related to various factors such as X-linked activation and skewing, to a higher number of miRNAs, hormonal variations, ischemic stroke, X-chromosome linked receptors such as G-proteins, interleukin and Toll-like receptors and variations in transcriptional and translational factors, thus explaining differences in the immune-response between sexes. A sex bias is found in infection and sepsis and sex-related to immune response to vaccination. Major autoimmune diseases also show a sex-related distribution in females and males. Regulatory inflammatory pathways are dependent on estrogen, androgen and testosterone levels. Sex-related diversity is found in human microbiota (skin, gut, lung, etc). There is complex regulation of the microbiome, hormones and genes, by estrogen, and this influences immune responses at many levels.

Professor Kathryn Sandberg, Georgetown University, Washington, DC, USA: How to reverse the trend? The NIH policies and recommendations from the Georgetown Consensus Conference Work Group

In mid January 2014, political awareness rose on gender bias in preclinical and basic research. In 2015 all stakeholders discussed and agreed that grant submissions to the National Institutes of Health (NIH) should also include sex as a biological variable. On 19 October 2015, J A Clayton published an article on guiding principles to consider sex as a biological variable in FASEB J.3 This will mostly affect preclinical research studying both sexes. In addition, the Georgetown consensus group with experts from different fields has proposed different strategies to promote research on sex and gender, to foster research on gender differences and establish new research institutes and promote educational training.

Dr Michael F W Festing, University of Leicester, Leicester, UK: Will we be able to reduce the number of animals if both sexes are used in research

Many studies use only male animals, but recent developments require grant applicants to balance male and female animals in preclinical and basic research projects. Several factors need to be considered such as the 3Rs, replication, randomization and the need for enough statistical power. Several possibilities are proposed: a factorial design allowing the use of 10 females and 10 males, rather than 20 animals of a single sex, with little difference in statistical power; use of analysis of variance (ANOVA) analysis and determine two-way interactions by a randomized block design or a preferential design in little blocks that allows interpretation using a multi-way ANOVA.

PD Dr Beat M Riederer, Editor-in-Chief, Laboratory Animals, UK: Sex-specific reporting of scientific research

Females have been neglected in biomedical research for a long time, leading to withdrawal of prescription drugs due to a sex bias in pain research and a bias in publishing scientific data. Two important points which may promote research that includes both genders are economics and ethics with regard to the 3Rs. Breeding only one sex versus breeding both females and males costs double and uses double the number of cages, which is especially true for transgenic animals. Publishing biomedical research in Laboratory Animals requires that the ARRIVE guidelines are respected and that species, strain and sex are mentioned and the number of animals used is justified. In addition, the use of only one sex needs to be mentioned and justified. Such information may provide valuable indications for the planning of preclinical studies. A recent editorial has addressed this issue.4

Professor Richard Svanbäck, Uppsala University, Uppsala, Sweden: Sex-dependent effects on microbiota

Vertebrates harbor diverse communities of symbiotic gut microbes. It has been shown that microbiota composition depends on interactions between host diet and sex within populations of wild and laboratory fish, laboratory mice and humans. Experimental diet manipulations in laboratory stickleback and mice have confirmed that diet affects microbiota differently in males versus females. The prevalence of such genotype and environment (sex by diet) interactions implies that therapies to treat dysbiosis might have sex-specific effects.

Professor Robert E Sorge, University of Alabama at Birmingham, Birmingham, USA: Sex and gender differences in pain and analgesia

Pain is subjective and sex-dependent. Women greatly outnumber men in reports of chronic pain. There are
known differences in receptor function and location between the sexes in addition to hormonal differences. There are clear sex differences with respect to immune system involvement in pain in animals, suggesting that the same might be true in humans. There is a significant sex difference in neuropathic pain in mice. The mechanisms of chronic pain in male mice involving microglia in a testosterone-dependent manner, while female mice utilize T-cells to mediate pain instead. These data translate to a variance in presentation and prevalence of chronic pain between men and women.

References
4. Riederer BM. Why sex is important in biomedical research. Lab Anim 2015; 49: 265–266.

Summarized by Beat M Riederer
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We are delighted to invite you to the 13th FELASA congress: 'Brussels Revisited' on 13-16 June 2016 in Brussels. Of course, Brussels is also the political centre of the European Union who have decided to innovate the Directive for the protection of animals used for research (2010/63 replacing 86/609). This new Directive aims to create a level playing field in Europe regarding the use of animals. The scale of the new Directive’s innovations reflects high ambitions to further develop and implement 3Rs alternatives and governance (by greater transparency). As a result, the Congress will also be of great interest to politicians and public bodies across Europe.

We are looking forward to welcoming you.

There will be state-of-the-art plenary presentations and your abstracts for oral presentations and posters are warmly welcomed (go to www.FELASA2016.eu for abstract submission).

The main themes will address:

- **Models by design**: Animals used for research typically represent a larger population. Sometimes, animals model for animals (e.g. new pharmaceutical formulas for veterinary use) but more often a suitable animal model is sought as a stand in. Also, developing technologies are used to improve and refine animal models in a way that the outcomes address mechanisms in addition to the effects of experimental approaches and the scientific questions they are to resolve. Nonetheless, experimental design is critical to acquire meaningful and reproducible results. Finally, we’re confronted with a wealth of scientific information in need of critical analysis and appropriate use.

- **One health**: The concept of ‘one health’ has been warmly adopted by the veterinary and human health communities alike. It is based on what animals and humans have in common, such as infectious diseases but also others like metabolic diseases and cancer. Similarly, animals and humans are exposed to the same environmental factors ranging from environmental pollutants to antimicrobials promoting microbial resistance.

- **What animals tell us**: Animals tell us a lot of things regarding their wellbeing. This stream addresses welfare topics to be analysed and addressed by increasing awareness on what’s going on in animals’ bodies and minds. The increasing significance of neuroscience research in animals, in part aiming to manage the increasing load of neural diseases in humans, can be used better to help address animal welfare issues as well.

- **On the safe side**: This stream will deal with the topics regarding animal and human safety. Human safety working with animals in laboratories, setting brings the needs for risk management. But also animals should be kept safe by the management of their health and wellbeing and procedures should follow best practices as these are developed over time. So, animal and human safety will be part of one stream that will also deal with new insights of facility design.

- **One Europe**: Europe is not just a geographical entity, it is an area of the world with shared values and common history. Politically, Europe is more or less unified by bodies such as the Council of Europe and the European Union. But also science and technology are developing by intense international collaboration. Hence strategies, methods and professional competences are merging. To capture that, this stream is on strategic scientific, technological, regulatory and human resources developments occurring internationally.

- **Governance**: Given the moral status of animals in our part of the world, there are genuine concerns in society about the instrumental use of animals in research. These concerns regard not only animal welfare but also the ethical justification of animal use. Bottom line, we need to explain our work and our attitude better to justify animal use in view of the real societal interests at stake. Public trust is to be maintained and gained by effective regulations and oversight, and above all by better communication. This is true for all areas of research but especially urgent in the case of animal use. Thus, this stream will be devoted to topics on ethics, oversight, accountability and public communication.
I'm a scientist and I actually care about the welfare of the animals I use. I'm interested to see what's considered best practice, also regarding experimental design.

I'm working for my government as a policy advisor and I'm keenly interested how we do nationally regarding the implementation of 2010/63.

I'm a senior technician and recently appointed as the one responsible for oversight on animal welfare in my department. I've also joined the Animal Welfare Body. I've the feeling I should strengthen my expertise and I'm also looking for training opportunities for my co-workers.

I'm a member of an ethical review board of a large academic institution and I want to have a bigger picture on the state of the art in experimental biomedicine.

Within the board of directors of a large company I'm responsible for our animal use. I'm deeply concerned about the publication of non-technical summaries. What can or should we do to inform the public better on our own account? And could we do something collectively with others?

I'm a veterinarian and I've worked for five years in a specialized companion animal practice where also small rodents and exotics were referred frequently. That was taken into account when I was employed as the designated veterinarian of a very diverse research institution. I need to know more about severity assessment and good experimental technique, as well as some workers' risks. And I've been tasked to set up a rehoming program for the cats we use in behavioural studies.

I've just started a PhD study also involving the use of animals. I'm interested in the practical and ethical aspects and would like to contribute to 3Rs. My supervisor will only let me go in case I've an abstract accepted for poster presentation.

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Obituary

Professor John Bleby, 1932–2015

Malcolm Gamble

We note with sadness the passing of John Bleby, one of the founding fathers of Laboratory Animal Science.

In the 1960s John succeeded Bill Lane-Petter as Director of the Laboratory Animals Bureau as it became the Medical Research Council (MRC) Laboratory Animals Centre (LAC) in Carshalton, Surrey, and he continued his pioneering work in improving the health and welfare of animals used in medical research. He established the LAC as the international centre for this work and his team of specialists founded the modern concepts of standardization for all aspects of animal husbandry. His team included Michael Festing and Dave Lovell in Genetics, Auriol Hill in Microbiology, Dawn Owen in Parasitology, David Ford in Nutrition, David Blackmore in Pathology, Gordon Townsend and Stephen Sparrow in Veterinary Services, Michael Robinson and Harry Donnelly in Accreditation, Gerald Clough and Malcolm Gamble in Environmental Physiology, and Terry Pendry and John Williamson in the animal houses and laboratories. The advances this team made, especially the establishment and availability of disease free (speci ed pathogen-free [SPF]) and germ-free stocks of rodents and animal house, cage and experimental design and care formed the basis of current practice in the ethical use of laboratory animals in medical and scienti c research.

As Professor at the Royal Veterinary College, he founded and organized the Master’s Degree course for vets in laboratory animal science, and long after many other people would have retired he started his own company assessing health pro les of laboratory animals. He was a former captain in the Royal Army Veterinary Corps (RAVC) Regular Army Reserve and a lieutenant-colonel in the RAVC’s own territorial army (TA) unit. He served on numerous veterinary and medical committees and councils. He was also the Food and Agriculture Organization’s veterinary consultant to developing countries and the World Health Organization’s veterinary consultant to the US National Institutes of Health and China.

He encouraged the establishment of the Institute of Animal Technicians (later Technology) and was one of its vice presidents for over 40 years.

He, together with his little book of appalling jokes, will be sadly missed. We wish Jayne and his family every best wish for the future.

Malcolm Gamble

October 2015

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The Animals (Scientific Procedures) Act, 1986 and the EU Directive 2010/63 both identify the need for laboratory animal care personnel to be formally recognised as being professionally qualified, including Higher Education Qualifications at levels 4 to 6. All these qualifications will therefore build on that need. They will deliver the knowledge to enable individuals to achieve a full and sustainable career pathway. For some, this may include the entry into Master's Degree programmes and to aspire to ever more senior positions.

I am confident that graduates from these courses will be able to make a very significant contribution in influencing the application of high standards of animal welfare, care and use in the UK Life Sciences. This will importantly include promoting and implementing the 3Rs (Replacement, Reduction and Refinement of animal use) in research facilities throughout the UK – something which the Government strongly advocates."

Dr Judy MacArthur Clark CBE MRCVS Head, Animals in Science Regulation Unit

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Calendar of events

Meetings of interest to laboratory animals scientists and technicians: references to Laboratory Animals are for further details. Items for inclusion should be sent to Notes and Comments Editor, LAL, PO Box 373, Eye, Suffolk, IP22 9BS, UK. Email to comments@lal.org.uk. The deadlines for inclusion of material are: February issue, 10 November; April issue, 10 January; June issue, 10 March; August issue, 10 May; October issue, 10 July; December issue, 10 September.

2016

1–3 February FRAME Training School in Experimental Design and Statistical Analysis, Voss, Norway. For further information visit http://www.frame.org.uk/training-schools/


15–16 February Life Sciences Switzerland Conference, Lausanne, Switzerland. For further information visit http://ls2-annual-meeting.ch/

2–4 March Evolutionary Systems Biology: From Model Organisms to Human Disease, Cambridge, UK. For further information visit https://registration.hinxton.wellcome.ac.uk/events/item.aspx?e=568


8–11 March Institute of Animal Technology Congress, Northern England. For further information visit http://www.iat.org.uk/#icongress/c16th

20–23 March 13th Transgenic Technology meeting, Prague, Czech Republic. For further information visit http://www.transtechsociety.org/tt2016/

22–23 March Fish Veterinary Society Annual Conference, Edinburgh, UK. For further information visit http://www.fishvetsociety.org.uk/

18–20 April Workshop: Assessment, Prevention and Alleviation of Pain and Distress in Laboratory Animals, Newcastle-upon-Tyne, UK. For further information visit ncl.ac.uk

20 April Establishment Licence Holders’ Spring Meeting, York, UK. For further information visit info@lasa.co.uk


13–16 June FELASA Congress, Brussels, Belgium. For further information visit http://www.felasa.eu/announcements/felasa-2016-in-brussels

16–17 June RSPCA International Meeting: Focus on severe suffering, Brussels, Belgium. For further information visit research.animals@rspca.org.uk

8–10 August 3rd World Congress on Pharmacology, Birmingham, UK. For further information visit http://pharmacology.pharmaceuticalconferences.com/


13–14 September 45th GV-SOLAS Seminar on Laboratory Animals and Animal Experimentation, Berlin, Germany. Further information to follow.

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