Universidad Autónoma de Tlaxcala

Centro Tlaxcala de Biología de la Conducta

Posgrado en Ciencias Biológicas

Termorregulación intra-camada en mamíferos altriciales: mecanismos y efectos a corto y mediano plazo

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QUE PARA OBTENER EL GRADO ACADÉMICO DE

DOCTOR EN CIENCIAS BIOLÓGICAS

P r e s e n t a

José Alfredo Zepeda Zempoaltecatl

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Tlaxcala, Tlax.   Julio, 2019

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Los abajo firmantes, miembros del jurado evaluador del proyecto de tesis que **José Alfredo Zepeda Zempoaltecatl** realiza para la obtención del grado de **Doctor en Ciencias Biológicas**, expresamos que, habiendo revisado la versión final del documento de tesis, damos la aprobación para que ésta sea impresa y defendida en el examen correspondiente. El título que llevará es **"Conducta termorregulatoria intracamada en mamíferos altriciales: mecanismos y efectos a corto y mediano plazo"**.

Sin otro particular, aprovechamos para enviarle un cordial saludo.

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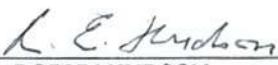
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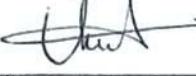
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RESUMEN

Las diferencias individuales en conducta y fisiología son en parte resultado de las interacciones entre hermanos que ocurren durante el desarrollo temprano. Para los mamíferos altriciales que crecen en camadas, la termorregulación conductual es uno de los contextos en donde las interacciones entre hermanos son relevantes para la ontogenia de las diferencias individuales. Desde hace más de medio siglo se ha estudiado el desarrollo de la termorregulación en este tipo de mamíferos. Por un lado, los trabajos en especies como la rata, el ratón y el conejo describen los beneficios y costos energéticos, y como repercuten en el crecimiento y desarrollo de los individuos. Por otro lado, estos trabajos, han develado los mecanismos fisiológicos y conductuales implicados durante la termorregulación y la edad en que las crías pueden alcanzar la independencia termorregulatoria. Sin embargo, durante la termorregulación conductual realizada por estas crías, ocurren interacciones tempranas entre los miembros de la camada, cuyos efectos sobre el desarrollo de las diferencias individuales aún están poco estudiados. Estas crías altriciales que nacen en camadas forman un agrupamiento dentro del nido para termorregular, el agrupamiento aporta beneficios energéticos a los individuos, que ayudan a mejorar su crecimiento y desarrollo. No obstante, dentro de la camada existe un aprovechamiento diferencial de esos beneficios. Uno de los principales factores que explica esta situación, es la existencia de una amplia variación individual en el peso al nacimiento. Para estos mamíferos, al igual que para otros grupos taxonómicos, el peso con el que nacen los individuos es un predictor importante de diferentes trayectorias de desarrollo. En estas crías de mamíferos el peso corporal dirige una combinación de mecanismos conductuales y fisiológicos que, para el individuo durante la termorregulación temprana, representan acceder a una posición térmicamente favorable al interior del agrupamiento.

En esta tesis investigamos los efectos a corto y mediano plazo de la interacción temprana entre hermanos sobre su crecimiento y desarrollo. Además, las relaciones causales entre los mecanismos implicados durante la termorregulación conductual en crías de mamíferos altriciales. Así mismo, investigamos la generalización de estos mecanismos y sus efectos, en dos especies de mamíferos: el conejo y el ratón doméstico.

Capítulo 1 *Body mass modulates huddling dynamics and body temperature profiles in rabbit pups*. Hasta ahora sabemos que las crías más ligeras de la camada están en contacto en promedio con un menor número de hermanos, ocupan las posiciones más periféricas dentro del agrupamiento, pierden más calor y tienen temperaturas corporales más bajas en comparación con el resto de sus hermanos. Sin embargo, estas crías periféricas invierten la mayor parte de su energía y tiempo desplegando conductas para acceder al centro del agrupamiento desde la periferia, a pesar de ello, constantemente su temperatura corporal es más baja que la de sus hermanos más centrales y pesados. Entonces, nos preguntamos ¿cómo la temperatura corporal es afectada por el cambio en el número de vecinos en contacto, producto de estos intentos por acceder al centro del agrupamiento? y ¿en qué grado este cambio en la temperatura corporal está explicada por las diferencias iniciales en el peso corporal de los individuos?

Nuestros resultados demostraron que la temperatura corporal superficial (determinada mediante termografía infrarroja) fue afectada significativamente por los cambios en el número de vecinos en contacto durante el agrupamiento; de hecho, estos cambios dependen en gran parte del peso relativo de las crías al interior de la camada. Este resultado nos permite entender cómo las diferencias individuales en el peso corporal intra-camada contribuyen a la emergencia de diferencias individuales entre hermanos durante sus interacciones en la vida temprana.

Capítulo 2 *Revealing the mechanisms of the association between body mass at birth and body mass at weaning in the domestic rabbit*. En especies de mamíferos altriciales que nacen en camadas con poco cuidado parental, agruparse con hermanos durante la vida temprana es una estrategia para mantener una temperatura corporal alta y estable. En este contexto, se ha enfatizado el significado de las diferencias individuales en la conducta de agrupamiento dentro de la camada. Aquellas crías que constantemente ocupan la posición central dentro del agrupamiento obtienen ventajas como, una temperatura corporal más alta y acceso rápido y prolongado a la leche de la madre durante el amamantamiento, todo esto contribuye a un mayor crecimiento en estos individuos. Mediante el uso de un análisis confirmatorio (*path analysis*) cuantificamos las relaciones causales entre estas variables. Nuestros resultados mostraron que

la interacción temprana en el agrupamiento dentro del nido, no sólo explica las diferencias individuales en crecimiento dentro de la camada, sino que también modifica la variación a nivel poblacional en el peso corporal al destete. Estos resultados, analizados en conjunto, confirman que las diferencias individuales, producto de la interacción entre hermanos durante el agrupamiento, desempeñan un papel clave al dar forma a la variación en crecimiento posnatal temprano, tanto intra-camada como a nivel poblacional.

Capítulo 3 *Individual differences in early body mass affect thermogenic performance and sibling interactions in litter huddles of the house mouse*. En el ratón doméstico nos preguntamos si, como ocurre en otras especies altriciales que hemos estudiado, las diferencias intra-camada en peso corporal en edades tempranas están asociadas con diferencias individuales en su desempeño termogénico y, además, si esa variación en peso predice diferencias individuales en la competencia por acceder a los sitios térmicamente más ventajosos dentro del agrupamiento. Nuestras predicciones confirmaron que aquellas crías relativamente más ligeras dentro del agrupamiento, estuvieron menos tiempo en contacto con sus hermanos y mostraron una pérdida de calor más pronunciada cuando fueron expuestas individualmente a temperaturas ambientales por debajo de su zona termoneutral, además de que consistentemente realizaron más conductas dirigidas (*rooting/climbing*) para acceder al sitio térmicamente más ventajoso del agrupamiento.

ÍNDICE

I. INTRODUCCIÓN

La termorregulación consiste en el mantenimiento de una temperatura corporal relativamente estable. Esta parte de la homeostasis es esencial para el funcionamiento de los tejidos del individuo y está controlada por el cerebro, principalmente por regiones del área preóptica del hipotálamo (Morrison, 2011). La temperatura corporal de los mamíferos oscila entre límites muy estrechos debido a que son organismos endotermos capaces de mantener su temperatura estable mediante una serie mecanismos fisiológicos y conductuales (Hill, 2004). Sin embargo, al nacer, las crías de mamíferos de patrón politoco se enfrentan a ambientes térmicos variables que comprometen su homeostasis. Por ejemplo, en condiciones naturales, las crías del conejo europeo pueden nacer en nidos que normalmente están a una temperatura ambiental alrededor de 4 º C (Rödel y cols. 2008a). Cuando son expuestas a bajas temperaturas ambientales, las crías dentro del nido forman un agrupamiento con los miembros de la camada para evitar la pérdida de calor. Este agrupamiento es una entidad dinámica (Alberts, 1978a) que otorga beneficios energéticos a los participantes (Gilbert y cols. 2009). No obstante, existen diferencias individuales que podrían estar afectando el modo en el que esos beneficios son adquiridos.

1.1 Termorregulación en crías de mamíferos

Los mamíferos placentarios típicamente mantienen una temperatura corporal central en promedio de 37º C cuando están en reposo y no están bajo estrés térmico (Hill, 2004). Sin embargo, los neonatos de mamíferos al nacer se enfrentan a condiciones térmicas variables y en muchos casos desfavorables. Para las crías de mamíferos, durante los primeros días de vida, es transcendental mantener su temperatura corporal estable, ya que permitirá que sus tejidos funcionen adecuadamente (Hill, 2004). Existe una gran variación con respecto a las capacidades termorreguladoras en estas crías. Por ejemplo, el uso de los mecanismos termogénicos dependerá de la condición de desarrollo sensorial, locomotor y nutricional, acorde al tipo de desarrollo posnatal que presentan las crías de mamíferos precociales y altriciales (Derrickson,

1992). Ambos tipos de crías, de manera distinta, utilizan mecanismos fisiológicos, que dependen del metabolismo del individuo; y mecanismos conductuales, definidos por el uso de desplazamientos dirigidos a encontrar una zona térmica favorable.

1.2 Mecanismos fisiológicos.

Entre los mecanismos fisiológicos de teremorregulación encontramos a la termogénesis por tiriteo, que consiste en la producción de calor resultante de la actividad metabólica necesaria para la contracción y relajación del músculo esquelético (Leon, 1986). De igual forma, encontramos a la termogénesis sin tiriteo, dada por la activación del tejido adiposo pardo (TAP) controlada por el sistema nervioso simpático, y por la vasoconstricción, que reduce la pérdida del calor metabólico por convección y radiación de la superficie de la piel (Kurz, 2008).

La termogénesis por activación del TAP es definida como la producción de calor mediante a la transformación de energía metabólica por procesos que no implican contracción del músculo esquelético (Tews y Wabitsch, 2011). En los neonatos y mamíferos pequeños, es definida como la producción de calor en respuesta a la variación en la temperatura ambiental para proteger al organismo contra la exposición al frío (Lowell y Spiegelman, 2000; Sessler, 2008). En el recién nacido, opera un mecanismo que acelera la beta-oxidación de grasas por activación del sistema nervioso simpático (Schmidt, 1985) en el TAP y es considerado como el principal centro termogénico en crías altriciales (Himms-Hagen, 1985; Hull y Segall, 1965).

1.3 Mecanismos conductuales.

Como parte de la termorregulación conductual, las crías son capaces de moverse hacia áreas más calientes disminuyendo la perdida de calor. Esto ha sido reportado en cerdos (Mount, 1963), gatos (Freeman y Rosenblatt, 1978), ratas (Kleitman y Satinoff, 1982), conejos (Kleitman y Satinoff, 1981; Pacheco-Cobos y cols. 2003) y ratones (Ogilvie y Stinson, 1966) por citar algunos. En estos trabajos los individuos, incluso desde los primeros días de nacidos, son capaces de moverse a través de un gradiente, siempre buscando temperaturas más cálidas. Por

supuesto en especies como ratas y ratones, esta capacidad para desplazarse hacia zonas más cálidas va mejorando con la edad, , como resultado de su desarrollo físico. No obstante, las necesidades térmicas son solventadas por el agrupamiento dentro del nido (Alberts, 1978a).

1.3.1 Conducta termorreguladora: agrupamiento.

La regulación conductual mediante el agrupamiento es considerada como un poderoso efector termorregulatorio. En los animales es utilizada para mantener el balance de calor respecto al ambiente (Sessler, 2008). El agrupamiento es una agregación establecida por la tendencia de las crías de aproximarse unas a otras y mantenerse en contacto (Schank y Alberts 1997). Es una conducta cooperativa controlada por señales sensoriales; táctiles, olfatorias y térmicas (Alberts, 1978b; Schank y Alberts, 1997). El agrupamiento es fundamental para conservar el calor metabólico (MacArthur y cols. 1997) y para reducir la pérdida de calor individual minimizando el área de superficie expuesta (Alberts, 1978a; Haig, 2008) como ocurre en crías de mamíferos altriciales al incrementar el contacto con sus compañeros de camada (Sokoloff y Blumberg, 2002). Por lo tanto, la habilidad de una cría para mantener su temperatura corporal depende de sus ajustes conductuales y fisiológicos (Sokoloff y Blumberg, 2002).

1.4 Termorregulación en crías de mamíferos precociales.

Las crías de mamíferos precociales nacen cubiertas con pelo, abren los ojos relativamente temprano y su desarrollo y crecimiento es acelerado en comparación con aquellas que son altriciales (Derrickson, 1992). Cuando están recién nacidos el principal mecanismo para regular su temperatura es el tiriteo, en ungulados como terneros (Carstens, 1994) y corderos (Bird y cols. 1998), y por vasoconstricción como en la liebre (Hackländer y cols. 2002). Por su parte, en especies de menor tamaño juega un papel más importante la termogénesis sin tiriteo mediante mecanismos endógenos como la activación del tejido adiposo pardo (Leon, 1986).

1.5 Termorregulación en crías de mamíferos altriciales.

En contraste con las crías de mamíferos precociales, las altriciales se caracterizan por sus capacidades físicas y fisiológicas limitadas para evitar la pérdida de calor. Debido a esto su desarrollo y supervivencia se encuentran comprometidos desde el momento de nacer. Sin embargo, en diferentes especies se ha caracterizado un rango de temperatura ambiental (zona termo-neutral; ZTN) dentro del cual la tasa metabólica basal es suficiente para reducir la perdida de calor (Gordon, 2012). Cuando las crías se exponen a temperaturas ambientales por arriba o por debajo de ese rango utilizan mecanismos para disipar el calor o bien para generarlo. Al nacimiento, el límite inferior para el conejo doméstico se encuentra alrededor de los 35 º C (Hull, 1965), en el ratón doméstico es de 35 º C después de los 13 días (Lagerspetz, 1962) y en la rata se encuentra igualmente alrededor de los 35 º C al nacimiento (Blumberg y Stolba, 1996).

Cuando las crías son expuestas temperaturas ambientales moderadamente por debajo del límite inferior de su ZTN, por un lado, elevan su producción de calor utilizando la activación del TAP, el cual juega un papel central en el incremento de la producción de calor cuando los neonatos son expuestos al frío (Dawkins y Hull, 1964). Por otro lado, conductualmente se agrupan con sus hermanos dentro del nido para reducir la pérdida de calor corporal al incrementar su aislamiento por contacto, con consecuencias positivas para su sobrevivencia y desarrollo (Alberts, 1978a; Bautista y cols. 2003; Gilbert y cols. 2007; Rödel y cols. 2008b; Harwsaw y Alberts, 2012).

1.5.1 Beneficios térmicos del agrupamiento en crías altriciales.

Dentro de los beneficios que otorga el agrupamiento dentro del nido a las crías altriciales se encuentran: reducir la pérdida de calor, disminuir el consumo energético y mejorar sus tasas de crecimiento. Por ejemplo, Alberts (1978a) evaluó el consumo de oxígeno en crías de rata que formaban un agrupamiento versus aquellas que se encontraban solas, determinó que el consumo de oxigeno es menor y pierden mucho menos calor aquellos individuos que estuvieron

agrupados. De igual manera, en el ratón doméstico, el consumo de oxígeno es menor en aquellos individuos que permanecen agrupados en comparación con aquellos que se encuentran aislados (Stanier, 1975), y la pérdida de calor es menor en aquellos individuos que se agrupan dentro del nido (Harshaw y Alberts, 2012). Por su parte, en el conejo doméstico cuando las crías son expuestas a temperaturas ambientales por debajo de su ZTN durante la primera semana de vida, los individuos que están agrupados tienen temperaturas corporales más altas (Bautista y cols. 2003). Además, aquellos que están aislados utilizan hasta un 40 % más de su energía para termorregular eficientemente comparados con individuos agrupados, (Gilbert y cols. 2007).

1.5.2 Ventajas del agrupamiento en crecimiento y supervivencia.

A pesar de que las crías altriciales desde el primer día de nacidas son capaces de generar calor mediante la activación del TAP, el agrupamiento es el principal mecanismo del que depende su termorregulación durante la primera semana de vida. El aislamiento térmico y la reducción del gasto energético que les otorga el agrupamiento se traducen en crecimiento y supervivencia para las crías. Por ejemplo, aquellas crías del conejo que permanecen solas durante la exposición a temperaturas ambientales por debajo de su ZTN, son menos eficientes en convertir la leche ingerida a biomasa, y por lo tanto crecen menos rápido en comparación con las que se mantienen agrupadas (Bautista y cols. 2003; Gilbert y cols. 2007; Nicolás y cols. 2011). No sólo crecen más, su desarrollo motor y el mantenimiento de su equilibrio corporal también es potenciado en las crías que permanecen agrupadas en comparación con aquellas que se criaron individualmente (Nicolás y cols. 2011). Mediante el agrupamiento, las crías defienden mejor su temperatura corporal, son capaces de destinar más energía al crecimiento y mejorar su desarrollo motor, como consecuencia tienen más posibilidades de sobrevivir. La supervivencia de las crías depende del aporte individual al bien común que genera el agrupamiento (Bautista y cols. 2003; Gilbert y cols. 2012).

1.5.3 Mecanismos que controlan la conducta termorregulatoria.

Para ratas, ratones y conejos, dentro de los mecanismos implicados en la conducta termorregulatoria se encuentran la orientación por pistas térmicas, olfativas y táctiles (Alberts, 1978b; Hull y Hull, 1982; Pacheco-Cobos y cols. 2003). Cuando las crías se mueven guiadas por estas pistas, el agrupamiento se torna en una entidad dinámica. En esta dinámica, los miembros de la camada se mueven para mejorar su índice de agrupamiento. Por ejemplo, en ratas (Alberts, 1978a) y ratones (Harshaw y Alberts, 2012) los individuos constantemente se están moviendo hacia el centro del agrupamiento cuando son expuestos a temperaturas ambientales por debajo de su ZTN o hacia la periferia cuando la temperatura ambiental supera su ZTN. Para el estudio de esta dinámica en el conejo doméstico (Bautista y cols. 2008) y la rata (Bautista y cols. 2009) se han descrito dos conductas que mejoran la posición de las crías dentro del agrupamiento. Estas son el *rooting* y *climbing*. La primera consiste en utilizar las patas traseras para enraizarse en el agrupamiento, y la segunda implica que las crías trepan sobre otras para acceder a una zona térmica más favorable dentro del agrupamiento. A pesar de que, en general, las crías altriciales obtienen beneficios al agruparse dentro del nido, existen diferencias individuales en la ejecución de estas conductas y en la obtención de esos beneficios con consecuencias a corto y largo plazo tanto para su crecimiento como para su supervivencia.

II. ANTECEDENTES

2.1 El conejo doméstico como modelo para estudiar la conducta termorregulatoria intra-camada y sus efectos sobre el desarrollo de las diferencias individuales en conducta y fisiología.

Estudiar las interacciones entre hermanos en edades tempranas, en condiciones naturales, es complicado debido a que éstas ocurren dentro del nido o madriguera, generalmente las madres ocultan sus nidos y cuidan de ellos de manera exhaustiva (Mock y Parker, 1997). Sin embargo, el conejo doméstico, debido a su peculiar conducta maternal, es un modelo que permite estudiar las relaciones entre los miembros de la camada sin alterar la relación madre-

crías (Drummond y cols. 2000; Bautista y cols. 2003, 2005). Tanto en vida silvestre como en el laboratorio, la coneja construye un nido con heno y pelaje que retira de sus flancos, donde aproximadamente después de 31 días de gestación pare a sus crías y tras haber parido a la última cría, cierra la madriguera para volver hasta después de 24 h para amamantarlas por aproximadamente 3 a 4 minutos (Zarrow y cols. 1965; Hudson y Distel, 1982; Jilge y Hudson, 2001). Durante el amamantamiento la madre típicamente permanece inmóvil sobre la camada y finaliza la sesión de amamantamiento dando un salto abrupto fuera de la caja-nido (Bautista y cols. 2005; Hudson y Distel, 1982). Durante la ausencia de la madre los recién nacidos forman un agrupamiento con sus hermanos dentro del nido (Hudson y Distel, 1982). Este patrón conductual ha permitido estudiar, en el laboratorio, las interacciones entre hermanos cuando se encuentran agrupados y explicar en qué grado la interacción temprana entre hermanos contribuye al desarrollo de diferencias individuales en fisiología y conducta a corto y largo plazo (Hudson y cols. 2011).

La principal diferencia individual que está presente desde el nacimiento en las crías del conejo es el peso corporal, y , tal como ocurre en otras especies de mamíferos altriciales, el peso corporal al nacimiento depende del tamaño de camada, los individuos nacidos en camadas grandes tienen menor peso en comparación con los que provienen de camadas más pequeñas (roedores: Mendl, 1988; gatos: Hudson y cols. 2009 y conejos: Drummond y cols. 2000).

Estas diferencias en peso al nacimiento están asociadas a diferencias en la conducta termorregulatoria dentro del agrupamiento, así, las crías más ligeras, en comparación con sus hermanas más pesadas, realizan más tiempo las conductas de *rooting* y *climbing* presumiblemente con la "motivación" de acceder al centro y mejorar así su índice de agrupamiento (Bautista y cols. 2008; Rödel y cols. 2008b; Bautista y cols. 2009). A pesar de que las crías ligeras están la mayor parte del tiempo realizando estos desplazamientos, ellas son las que en promedio están en contacto con un número menor de hermanos dentro del agrupamiento, es decir permanecen en la periferia del agrupamiento más tiempo que sus hermanas relativamente más pesadas (Bautista y cols. 2013; Bautista y cols. 2015; Hudson y cols. 2011; Reyes-Meza y cols. 2011).

2.1.1 La posición ocupada por las crías dentro del agrupamiento modula las diferencias en fisiología, desarrollo y supervivencia.

Para las crías que nacen ligeras dentro de una camada del conejo doméstico la supervivencia y desarrollo están comprometidas. En primer lugar, debido a la pérdida de calor constante por su desfavorable razón superficie/volumen (Hill, 2004). En mamíferos de tallas pequeñas como las crías ligeras, la pérdida de calor es mayor, debido a que la cantidad de calor generado por sus pequeños cuerpos es mucho menor que el calor que pierde debido a su mayor superficie expuesta al ambiente.

En segundo lugar, las crías ligeras tienen un menor número de hermanos en contacto dentro del agrupamiento, ocupan las posiciones más periféricas, su temperatura corporal es menor, obtienen menor cantidad de leche durante el evento de succión (Bautista y cols. 2005) y como consecuencia de ello, ganan menos peso y sobreviven menos durante las primeras semanas de vida (Drummond y cols. 2000).

En tercer lugar, a pesar de que como muchos mamíferos, cuentan con reservas energéticas de tejido adiposo pardo que se encargan de la termogénesis no mediada por tiriteo (Dawkins y Hull, 1964; Kajimura y Saito, 2014) las crías más ligeras tienen que destinar más energía a la termogénesis en lugar de destinarla a crecimiento. En contraste con las crías más pesadas, cuando las crías ligeras son expuestas a temperaturas ambientales por debajo de su zona termoneutral, activan más su tejido adiposo pardo, presentan una expresión mayor de la proteína desacoplante número 1 UCP1 (Bautista y cols. 2013), y agotan sus reservas de ácidos grasos antes que sus hermanas más pesadas (García-Torres y cols. 2015) comprometiendo su crecimiento y posterior supervivencia.

2.2 El ratón doméstico como modelo para estudiar la conducta termorregulatoria intra-camada y su efecto sobre el desarrollo de las diferencias individuales en conducta y fisiología.

El ratón doméstico (*Mus musculus*) es la especie de la familia de roedores con mayor distribución geográfica, considerada ahora como una especie comensal del hombre, transportada accidentalmente desde Asia y Europa hacia el resto del mundo. Las crías de *Mus musculus* nacen desnudas y sordas dado su carácter altricial. El primer ambiente posnatal al que se enfrentan es un nido aislado construido con pelo, plumas y vegetación en condiciones naturales, y con papel y cartón en condiciones de laboratorio (Berry y Bronson, 1992). Durante las primeras semanas en el nido su ambiente social temprano está formado por sus hermanos que pueden ser de 4 a 9 y su madre. Conforme pasan los días se van sumando el padre (macho dominante) y algunos hermanos que no han alcanzado la independencia (Berry y Bronson, 1992). Desde el nacimiento hasta las 2 o 3 semanas la regulación de su temperatura depende de la madre y de sus hermanos, con quienes forman un agrupamiento dinámico en el cual, como ocurre en ratas y conejos, los individuos constantemente intentan llegar al lugar térmicamente más ventajoso (Harshaw y Alberts, 2012; Harshaw y cols. 2014; Latham y Mason, 2004). Sin embargo, no existen estudios que se enfoquen en el desarrollo de las diferencias individuales durante esta etapa crítica de la vida tempana.

III. CONTRIBUCIÓN DE LA PRESENTE TESIS

Con base en estos antecedentes nos preguntamos lo siguiente: en el conejo doméstico (*Oryctolagus cuniculus*; Capítulo I y II) ¿el aumento en la temperatura corporal, resultado de sumar hermanos en contacto durante el agrupamiento dentro de la camada, está asociado a diferencias en el peso corporal temprano? ¿cuál es la relación causal entre los mecanismos que se establecen desde el nacimiento hasta el desarrollo temprano para producir diferencias individuales entre las crías del conejo doméstico durante la primera semana de vida? ¿en qué

grado los mecanismos implicados en la primera semana de vida modulan la variación general en crecimiento hasta el destete? y ¿estos mecanismos, a pesar de la domesticación en condiciones de laboratorio, son preponderantes como ocurre en condiciones naturales?

Para ampliar el conocimiento sobre la ontogenia de las diferencias individuales en los mamíferos altriciales, realizamos un estudio comparativo con el ratón doméstico descendiente de una población silvestre como modelo (Capítulo III). En *Mus musculus* no se ha descrito la interacción entre hermanos durante los primeros días posnatales, específicamente las diferencias individuales presentes durante el agrupamiento que forman los miembros de la camada para termorregular. Es por eso, que nos preguntamos si, al igual que sucede en conejos, ¿existen diferencias entre los individuos al ocupar posiciones térmicamente ventajosas dentro del agrupamiento y si estas diferencias son un efecto del peso corporal en edades tempranas? De igual modo, ¿sí el desempeño termogénico individual es modulado por las diferencias individuales en peso corporal intra-camada?

IV. HIPÓTESIS GENERAL

En crías altriciales de patrón politoco, la interacción temprana entre hermanos durante el agrupamiento de la camada está mediada por mecanismos fisiológicos y conductuales que modulan el desarrollo de diferencias individuales en conducta y fisiología a corto y mediano plazo.

4.1. Hipótesis 1.

En los individuos relativamente más ligeros de camadas del conejo doméstico, los cambios en el número de hermanos en contacto, producto de sumar 1 ó 2 durante el agrupamiento dentro de la camada, se asocian a cambios en la temperatura corporal.

4.2. Hipótesis 2.

Las diferencias individuales en crecimiento en la primera semana de vida son moduladas por el peso al nacimiento mediante diferencias individuales en la posición ocupada dentro del agrupamiento. Al mismo tiempo, estas diferencias individuales intra-camada son un efecto indirecto del peso al nacimiento y contribuyen a explicar la variación en crecimiento cerca del destete entre-camadas del conejo doméstico europeo.

4.3. Hipótesis 3.

Las diferencias individuales intra-camada en el peso corporal temprano están asociadas a diferencias en el desempeño termorregulatorio en crías del ratón doméstico, así mismo, esta variación predice las diferencias individuales durante la competencia activa por los sitios térmicamente ventajosos dentro del agrupamiento de la camada.

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VI. Capítulo 1: Body mass modulates huddling dynamics and body temperature profiles in rabbit pups.

En el conejo doméstico, el tamaño de camada y el peso con el que nacen las crías guardan una relación negativa (Drummond y cols. 2000; Rödel y cols. 2008a). Esta variación inicial en el peso de las crías es uno de los factores que afectan el desarrollo de las interacciones tempranas dentro del agrupamiento (Bautista y cols. 2008; Rödel y cols. 2008b, Bautista y cols. 2015). Estas diferencias individuales en el peso al nacimiento están asociadas positivamente al número de hermanos en contacto (posición ocupada dentro del agrupamiento de la camada), la temperatura corporal, a la ingesta de leche, crecimiento y supervivencia. Si consideramos a la variación inicial en peso como el factor que dirige lo que ocurrirá después del nacimiento, podemos esperar que los individuos ocupen sus propios micro-nichos térmicos dentro del agrupamiento de la camada utilizando diferentes mecanismos termorregulatorios tanto fisiológicos como conductuales en función de su peso corporal.

Debido a lo anterior, en el Capítulo 1, nos interesó evaluar en el conejo doméstico la existencia de micro-nichos térmicos mediante termografías *in situ* sin alterar el agrupamiento de la camada. Previamente, hemos reportado que existen diferencias en la temperatura corporal entre crías de diferente peso corporal. Sin embargo, con esta nueva metodología podemos estudiar las diferencias en su respuesta termogénica sin alterar la dinámica del agrupamiento.

El trabajo que a continuación se presenta se publicó a partir de los datos generados para una investigación previa y de la obtención, análisis e interpretación de termografías tomadas durante distintas horas del día en camadas del conejo doméstico. Los resultados mostraron que durante el agrupamiento, existen micro nichos asociados a las diferencias individuales en peso corporal. Aquellos individuos más ligeros de la camada incrementan su temperatura corporal períferica incluso cuando aumentan en uno su numero de vecinos en contacto. Esto no tiene tanto efecto en las crías intermedias y pesadas de la camada, para quienes aumentar o disminuir su numero de vecinos en contacto no representa un incremento significativo en su temperatura corporal periferica.

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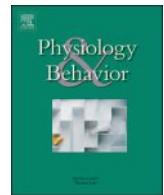
Body mass modulates huddling dynamics and body temperature profiles in rabbit pups

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ABSTRACT

Altricial mammals typically lack the physiological capacity to thermoregulate independently during the early postnatal period, and in litter-bearing species the young benefit strongly from huddling together with their litter siblings. Such litter huddles are highly dynamic systems, often characterized by competition for energetically favorable, central positions. In the present study, carried out in domestic rabbits *Oryctolagus cuniculus*, we asked whether individual differences in body mass affect changes in body temperature during changes in the position within the huddle. We predicted that pups with relatively lower body mass should be more affected by such changes arising from huddle dynamics in comparison to heavier ones. Changes in pups' maximum body surface temperature (determined by infrared thermography) were significantly affected by changes in the number of their neighbors in the litter huddle, and indeed these temperature changes largely depended on the pups' body mass relative to their litter siblings. Lighter pups showed significant increases in their maximum body surface temperature when their number of huddling partners increased by one or two siblings whereas pups with intermediate or heavier body mass did not show such significant increases in maximum body temperature when experiencing such changes. A similar pattern was found with respect to average body surface temperature. This strong link between changes in the number of huddling partners and body surface temperature in lighter pups might, on the one hand, arise from a higher vulnerability of such pups due to their less favorable body surface area-to-volume ratio. On the other hand, as lighter pups generally had fewer neighbors than heavier ones and thus typically a comparatively smaller body surface in contact with siblings, they potentially had more to gain from increasing their number of neighbors. The present findings might help to understand how individual differences in body mass within a litter lead to the emergence of individual differences in sibling interactions during early postnatal life in different species of altricial and litter-bearing mammals.

1. Introduction

One of the most important challenges facing newborn mammals is the need to maintain an adequate body temperature for survival and for (often rapid) growth. For altricial young born without fur, with a high body surface area-to-volume ratio, and without the ability for shivering thermogenesis, the challenge is particularly great [1–3]. Although many altricial mammals are born into the shelter of a den, nursery burrow or nest, during the mother's absence and periods of low ambient temperature they depend critically on behavioral thermoregulation such as huddling with littermates [4–8].

The European rabbit (*Oryctolagus cuniculus*), including its domesticated (laboratory) form, is a good example. The altricial, naked young are born into a nest of grass and fur constructed by the mother in a nursery burrow (or laboratory nest box) [9,10]. Mothers leave the young almost immediately after giving birth and only return for a few minutes approximately once every 24 h to nurse [11–14]. At around postnatal days 18–20, under natural conditions, the young emerge from the nursery burrow and are typically weaned during the following 10 days [15,16]. This means that rabbit pups spend the nest period almost exclusively in the company of their littermates, and that relations among them can be studied largely without potentially confounding maternal effects.

In the wild, ambient temperatures in the nest burrow can be

considerably lower than the newborn pups' critical thermoneutral temperature of approximately 35 °C [17]. The first seasonal litters in early spring can develop and survive even when the temperature of the soil at a depth where the nursery burrows are situated is lower than 5 °C [18]. From birth the pups perceive fine differences in environmental temperatures, and when placed on a thermal gradient quickly locate and come to rest in their thermoneutral zone [19–22]. The newborn pups depend critically on the presence of littermates for warmth and insulation, with implications for their growth and survival [6–8,18,23], and like the young of other altricial species they form a tight, insulating huddle at low ambient temperatures [4,5,24].

Typically, a litter huddle is not a homogenous system, and the central area is frequently better insulated and warmer than the periphery [25]. Thus, central and energetically more advantageous positions should be occupied by more competitively successful individuals, leading to a skew in the benefit of huddling among littermates. This has been shown, for example, in the European rabbit: Although the dynamics of a litter huddle are typically characterized by frequent changes in the positions of its members (see also [4,26] for similar behavior in the laboratory rat *Rattus norvegicus*), individuals with a higher body mass at birth relative to their litter siblings show a higher probability to consistently occupy more central positions ([27,28], see [29] for similar findings in laboratory rats). Rabbit pups occupying more central positions within the huddle also maintain higher body temperatures, obtain more milk, are more efficient at converting it to body mass, expend less time (and presumably energy) struggling to obtain such positions, and consequently show faster growth and have a higher probability of survival than their lighter, more peripheral sibs [25,30]. In a recent cross-fostering study in domestic rabbits we could show that these differences are due to relative differences in body mass among littermates rather than to genetic effects possibly associated with body mass at birth [28].

Despite the considerable information on huddle structure and dynamics in the rabbit and other altricial mammals, little is known about possible differences among individual littermates concerning the consequences of such dynamics for thermoregulation, and almost nothing in situ without disturbing the established huddle structure. In the present study we therefore examined in more detail the relation between differences among domestic rabbit littermates in body mass, the dynamics of changes in their position within the litter huddle, and associated fine scale changes in their body temperature. To this end we applied non-invasive measurements of body surface temperatures using infrared-thermography in undisturbed litters. In doing so we aimed to provide a more detailed understanding of the emergence of stable individual differences in behavioral and physiological profiles among littermates during the early developmental period, which is critical for adequate growth and survival in the European rabbit as well as in other litter-bearing altricial mammals [23,31–34]. We predicted that pups with relatively lower body mass should benefit more from increases in the number of neighbors in comparison to heavier ones. For this, we analyzed material from a previous study but which was conducted for a different purpose, namely to demonstrate by cross-fostering pups that differences among littermates in relative body mass (rather than absolute body mass or possibly associated (*epi*)genetic factors) can account for the previously reported strong relation between body mass at birth and intralitter differences in subsequent growth and survival [28]. Although based on the same animals, the analyses and findings presented here are new and have not been previously published.

2. Material and methods

2.1. Animals

We used a total of 15 litters of chinchilla-breed domestic rabbits culled to 8 pups each (*n* = 120 pups), as this is the maximum litter size for which we have found all pups to reliably survive the early postnatal

period. Chinchilla-breed rabbits typically have four pairs of nipples. Nevertheless, the sample size decreased to *n* = 112 pups by the end of the study (day 6) due to pup mortality. Deaths occurred in 7 of the 15 litters, all after postnatal day 3. Litters were from 15 different multiparous females each mated with one of 8 different stud males, bred and maintained at the Centro Tlaxcala de Biología de la Conducta (details in [28]). Females were kept in individual stainless steel cages 90 × 60 × 40 cm high and under fluorescent lights set to a 16:8 h light:dark cycle, which approximates conditions at the height of the summer breeding season for rabbits in Europe. Ambient air temperature was maintained between 17 and 24 °C, and water and food (Purina rabbit chow, Purina®, USA) were continuously available. On day 28 of gestation, 3 days before parturition, mothers were given hay and an open-top wooden box (40 × 35 × 15 cm high) in which to build a nest.

2.2. Procedure

Experimental animals were kept and treated according to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, USA, and the National Guide for the Production, Care and Use of Laboratory Animals, Mexico (Norma Oficial Mexicana NOM-062-200-1999).

2.2.1. Postnatal day 0

For the purpose of the previous study using these animals [28], on the morning of the scheduled day of birth (gestation day 31) we induced parturition by injecting the pregnant females with 5 IU (1 ml, i.m.) of oxytocin (Syntocinon, Basel, Switzerland). Immediately after the mothers had given birth (approximately 8 min after oxytocin injection) the boxes with the pups were removed from the mothers' cages, the pups were weighed on electronic scales (Ohaus Corporation, Scout Pro; Pine Brook, NJ, USA), and the litters culled to the 8 heaviest pups. The litters were then returned in the nest boxes to the mothers until the following day so as to allow at least one nursing episode without human disturbance.

2.2.2. Postnatal days 1 to 6

As in previous studies [27,28], at 09:00 h the following day the pups (dark gray in this breed) were taken from the mothers' cage, and identifying labels (numbers or letters) were painted on their sides and back with white correcting fluid and on their ventrum with black ink for individual identification in video recordings. They were then placed in a flannel-lined nest box but without nest material to allow behavioral recording. To prevent pups becoming trapped in the corners they were placed inside a 28-cm diameter wire mesh hoop. The box was placed in continuous light in a cool room at 25 °C, and thus below the thermoneutral zone for new-born rabbits of about 35 °C [17]. This induces pups to huddle but is not so low as to threaten their survival [6,18]. To ensure a thermal challenge to the huddle despite pups' increasing body volume and growth of fur from postnatal days 1 to 6, the ambient temperature was lowered by 1 °C each day after the last daily measurement of body temperature. An acrylic cover was placed over the box to protect the pups from draughts. After nursing on the morning of day 7 we returned the pups permanently to the mothers' cages to be raised to weaning without further disturbance.

2.3. Weighing of pups and definition of body mass categories

On postnatal days 2 to 6 at 11:00 h the pups were individually weighed, returned to the nest box, and the mothers were brought to them for nursing. For this the mesh hoop was removed without disturbing the huddle, and the mother was placed beside the box so that she could jump in for the approximately 3-min daily nursing [35,36].

Within each litter we defined body mass categories separately for each day, based on the weights obtained in the morning of postnatal days 2, 4 and 6. For this, we assigned individuals with a body mass

within the 33% lower percentile within each litter to the category "light", individuals with a body mass within the 33% intermediate percentile to the category "intermediate", and individuals with a body mass within the 33% upper percentile to the category "heavy". Note that there was high consistency over time with respect to individual body mass categories within litters as has been previously reported for the European rabbit (at least) during the early postnatal period (from postnatal days 0 to16 [30]). Distributions of the absolute body masses of the pups assigned to the different body mass categories are given in the supplementary material (Fig. A).

2.4. Determination of number of neighbors in the litter huddle

To measure pups' positions within the litter huddle relative to their littermates we analyzed the photographs corresponding to each thermal image (postnatal days 2, 4 and 6; 4 frames per day, see description below). For each pup in each frame we recorded the number of littermates ("neighbors") with which it was in contact with an estimated 10% or more of its body surface [35–37].

2.5. Collection of temperature data

On days 2, 4 and 6, the acrylic covers of the boxes containing the litters were briefly removed at 09:00, 13:00, 15:00 and 17:00 h, and at each of these times 4 snap shots of each litter were made using a handheld thermographic camera (Fluke Ti25, Fluke Inc. Everett, WA, USA; resolution: 640 × 480 pixels, thermal sensitivity < 0.1 °C) without disturbing the litter huddle (Fig. 1). For the analysis, only the clearest of each set of 4 snap shots was used. Although skin temperature cannot be considered an accurate measure of core temperature [5] it gives consistent recordings of relative differences in body temperature among littermates [6,7,36,38], which was the main interest in the present study.

For analysis of thermal images we used the software SmartView version 3.15.20.0 (Fluke Thermography, Plymouth, MN, USA). For this we traced the visible body contours of all pups within the litter huddle and for each pup we calculated the average and the maximum body surface temperature of the visible area (see Fig. 1). Note that the maximum body surface temperature of rabbit pups in this age range from 2 to 6 days (i.e., younger than 10 days, when young rabbits start to be well furred and to open their eyes) is usually situated at the intrascapular region, the main site of brown adipose tissue thermogenesis [39]. In cases where pups, and particularly their inter-scapular region,

were strongly overlapped by the body of another sibling, we chose a further, subsequent frame from the same session to obtain information on the temperature profile of that specific pup. In this way we obtained one (sometimes composite) record of individual differences among littermates for each of the four daily photographic sessions (in total 12 records per pup and litter).

Based on these data we calculated individual-level changes in body surface temperatures (both averaged and maximum body surface temperatures) for all cases where the number of neighbors (see definition above) remained constant (change = 0), or increased by one (change = 1) or by two neighbors (change = 2) between two different measurements within a day. All 6 possible comparisons for each of the 4 frames per day were done. Only increases were considered, as decreases would simply be the redundant inverse of the increases. Note that the different individual body surface temperature measurements within a day were always at least 2 h apart, and thus could be considered to be independent, as they did not reflect a sequence of continuous behaviors. In this way, we obtained a total of 497 cases where N neighbors stayed constant, 344 cases where N neighbors increased by 1 and 139 cases when N neighbors increased by 2.

2.6. Statistical analysis

Statistical analyses were performed with R version 3.4.0 [40]. We first calculated repeatability in the number of neighbors individual pups had within their litter huddle across the different measurements from days 2 to 6 (12 measurements per individual). We applied intra-class correlations (intra-class correlation coefficient: R_{ICC}) calculated as the proportion of phenotypic variation that could be attributed to between-subject variation [41], and to this end we used LMM-based calculations of repeatability based on the R package *rptR* [42]. We used individual identity as a random factor, and we corrected the model for potentially confounding effects of sex (fixed factor with 2 levels) and litter identity (random factor). *P*-values were calculated by 1000 permutations.

Furthermore, we tested for differences among body mass categories (factor with 3 levels: low, intermediate, high) with respect to the following response variables (different statistical models): the number of neighbors (see Fig. 2a), the within-litter differences in body surface temperature (mean and maximum temperature; see Fig. 2b, c) and the changes in body surface temperatures (mean and maximum temperature; see Fig. 3). Within-litter differences in body surface temperature were calculated as the deviation from the litter mean [cf. 38]. These different models also included the effects of sex (factor with 2 levels)
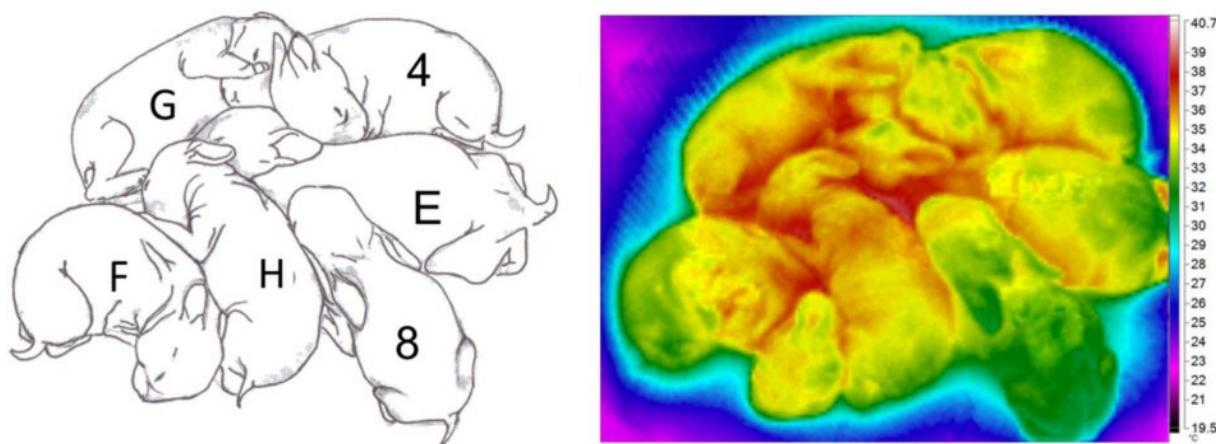

Fig. 1. An experimental litter of six surviving pups on postnatal day 6, marked for individual identification as reported in a previous study [28]. Left: an outline of the pups' positions in the litter huddle made from the photographic image corresponding to the thermal image (right), showing the number of littermates with which each pup was in body contact with at least 10% of its body surface. Pup E had the greatest body mass (133.1 g) and was in body contact with four littermates, while pup 8 with the lowest body mass (83.8 g) was in contact with two pups. Pup G with intermediate body mass (100.6) was in contact with three pups. Right: the corresponding thermographic image where red represents highest body surface temperatures grading to green representing lowest temperatures. Note the generally higher temperatures of more centrally located pups in body contact with several littermates compared with more peripheral pups in body contact with fewer littermates. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
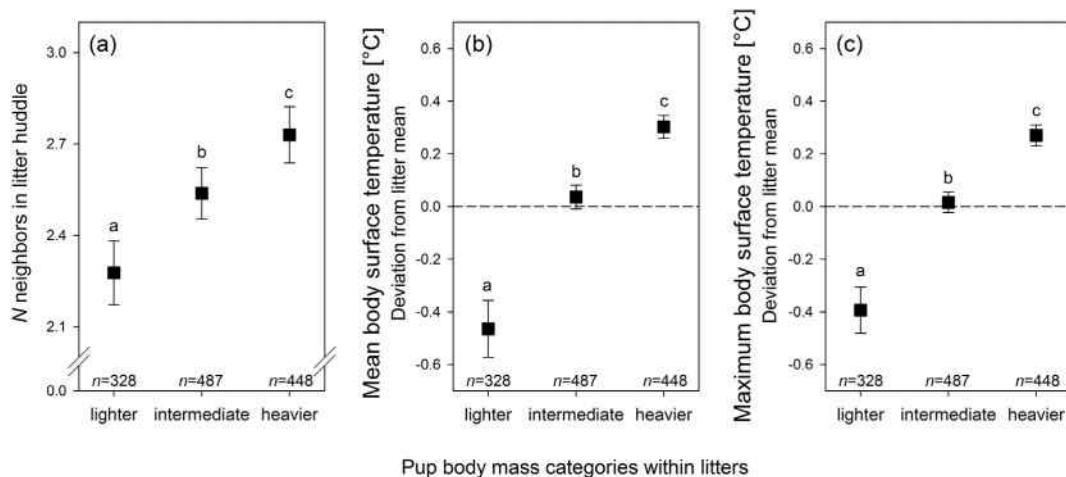
Fig. 2. Comparison of the number of littermates with which individual pups were in body contact ("neighbors") (a), and within-litter differences (deviation from litter means) in the mean (b) and maximum (c) body surface temperatures, between lighter (33.3% lower percentile), intermediate (33.3% intermediate percentile) and heavier pups (33.3% upper percentile) within litter huddles of domestic rabbits ($n_{litters}$ = 15). Means ± 95% confidence intervals are given. Litter size of 8 pups; in a few cases 1–2 pups per litter died in the course of the study. Repeated measurements (postnatal days 2, 4 and 6; 4 measurements per day) from 120 pups were analyzed using LMMs; see text for details of statistics. Sample sizes ($n_{measurements}$) and results of post-hoc comparisons (after sequential Bonferroni correction; significant pair-wise comparisons are indicated by different letters) are given in the graphs.

and of postnatal day (factor with 3 levels). Analyses were done by multifactorial linear mixed-effects models (LMM) using the R package *lme4* [43]. Models included two random factors (random intercepts): litter identity, as interactions within different litters were analyzed, and individual (pup) identity. The latter random factor was included as pups were repeatedly measured within and across postnatal days 2, 4 and 6. For *P*-value calculation we used conditional *F*-tests based on the Satterthwaite's approximation [44]. We checked all models for homogeneity of variances by plotting residual versus fitted values, and we verified that model residuals corresponded to a normal distribution using normal probability plots [45]. Pairwise post hoc comparisons for significant LMMs were carried out using LMM models of the same structure but based on a subset of the data including only 2 body mass categories (see Fig. 2, 3). Alpha levels (0.05) were Bonferroni-corrected by a sequential correction after Holm [46].

3. Results

3.1. Numbers of neighbors in the litter huddle and relative body surface temperatures: repeatability and association with body mass

The number of neighbors which individual pups had in their litter huddle from postnatal days 2 to 6 (R_{ICC} = 0.013, *P* = 0.020), as well as their relative mean (R_{ICC} = 0.055, *P* < 0.001) and average body temperatures with respect to their litter siblings (R_{ICC} = 0.070, *P* < 0.001) were significantly repeatable across time. In all cases, however, the intra-class correlation coefficients were rather low.

Pups' number of surrounding neighbors in the litter huddle increased significantly with an increase in their relative, intra-litter body mass category ($F_{2,972}$ = 20.08, *P* < 0.001). Accordingly, post hoc comparisons reported significant differences between lighter, intermediate and heavier pups (Fig. 2a).

Furthermore, pups' relative difference with respect to their litter siblings in their mean body surface temperature ($F_{2,1246}$ = 116.88, *P* < 0.001) as well as in their maximum body surface temperature ($F_{2,1246}$ = 124.03, *P* < 0.001) increased significantly with their relative intra-litter body mass category. Post hoc comparisons between lighter, intermediate and heavier pups are given in Fig. 2b, c. Such statistically significant differences were also apparent with respect to the absolute body surface temperatures (mean temperature: $F_{2,424}$ = 47.60, *P* < 0.001; maximum temperature: $F_{2,411}$ = 41.97, *P* < 0.001), which both increased significantly with the pups' relative intra-litter body mass category (see post hoc comparisons in the

supplementary material in Fig. B).

There were no statistically significant effects of sex or postnatal day on the number of neighbors or on the mean or maximum body surface temperatures (all *P* > 0.10).

3.2. Increase in number of huddling partners and associated body temperature changes

Lighter pups, for which the number of neighbors in the litter huddle increased even by just one sibling, experienced a significant increase in their mean ($F_{2,258}$ = 7.30, *P* = 0.001; post hoc comparisons in Fig. 3a) as well as in their maximum body surface temperature ($F_{2,257}$ = 8.71, *P* < 0.001; post hoc comparisons in Fig. 3d) when compared with situations in which no change in the number of neighbors occurred. Similar effects were observed in pups of intermediate body mass, which experienced a significant increase in their mean ($F_{2,373}$ = 6.62, *P* = 0.001; post hoc comparisons in Fig. 3b), but not in their maximum body surface temperature ($F_{2,375}$ = 0.70, *P* = 0.496; Fig. 3e) when the number of neighbors increased by two. In heavier pups, there were no significant differences between situations of no change in the number of neighbors or in an increase by one or two neighbors with respect to changes in pups' mean ($F_{2,333}$ = 2.62, *P* = 0.075; Fig. 3c) or their maximum body surface temperature ($F_{2,333}$ = 1.36, *P* = 0.258; Fig. 3f). There were no statistically significant effects of sex or postnatal day with respect to any of the changes in body surface temperature tested (all *P* > 0.10).

Furthermore, we conducted a comparison of the changes in body surface temperature resulting from changes in the number of neighbors between the three body mass categories within litters. This comparison revealed that the changes in the maximum body surface temperature in response to an increase by 2 neighbors were significantly higher in lighter pups compared to intermediate ($F_{1,79}$ = 4.95, *P* = 0.029; Fig. 3d, e) and heavier ones ($F_{1,73}$ = 5.25, *P* = 0.025; Fig. 3d, f). All other comparisons, also with respect to the mean body surface temperature, were not statistically significant (all *P* > 0.05).

4. Discussion

Our study reveals a differential advantage of the increase in the number of littermates with which individual rabbit pups have body contact ("neighbors") within the litter huddle, dependent on their relative postnatal body mass. During the first postnatal days an increase in the number of neighbors by just one or by two individuals led to
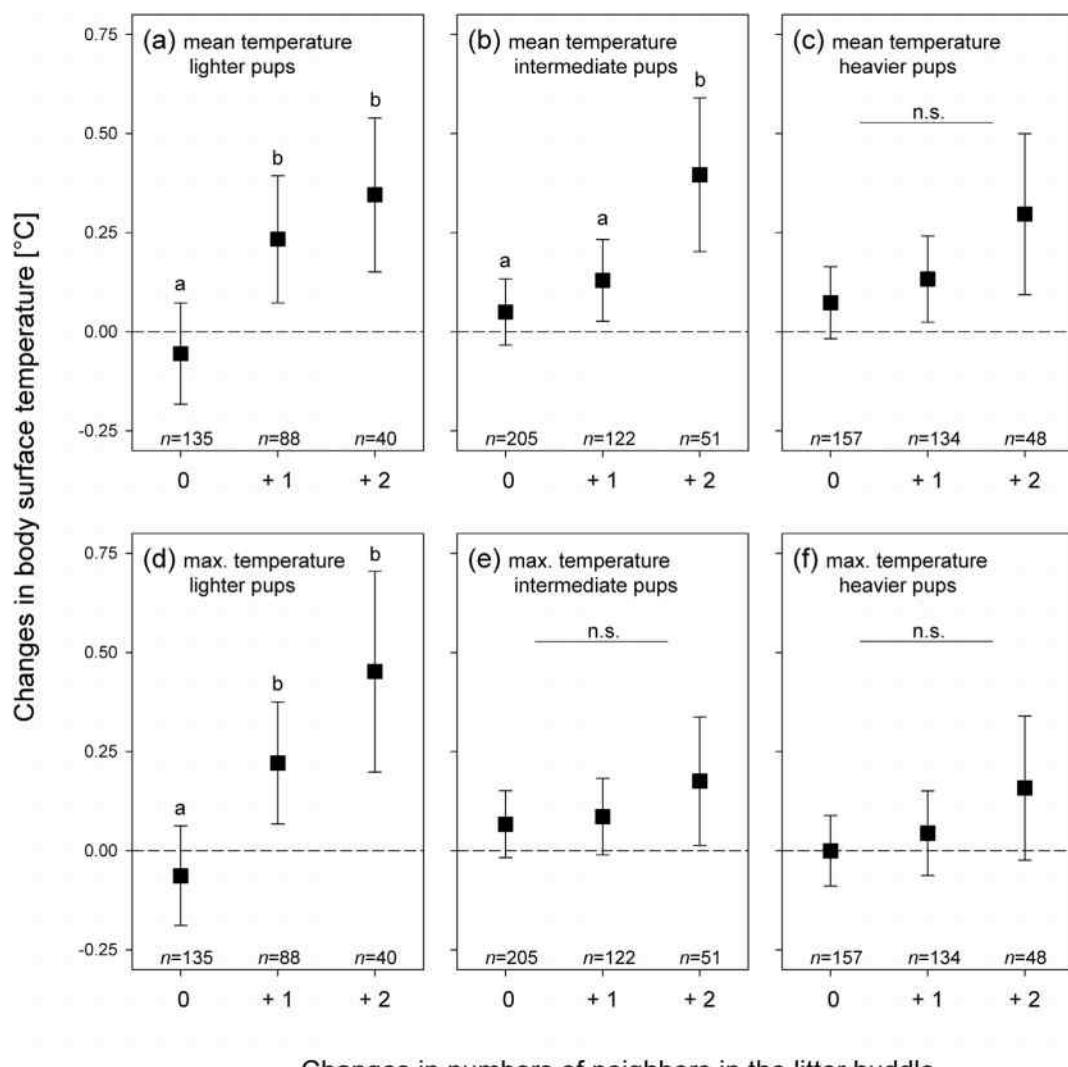
Fig. 3. Comparison of changes in the average body surface temperature (a, b, c) and changes in the maximum body surface temperature (d, e, f) of lighter, intermediate and heavier pups within litters of domestic rabbits ($n_{litters}$ = 15), assessed by infrared thermography. Means ± 95% confidence intervals are given. Litter size of 8 pups; in a few cases 1–2 pups per litter died in the course of the study. Repeated measurements (postnatal days 2, 4 and 6; 4 measurements per day) from 120 pups were analyzed using LMMs; see text for details of statistics. Sample sizes ($n_{measurements}$) and results of post-hoc comparisons (after sequential Bonferroni correction; significant pair-wise comparisons are indicated by different letters) are given in the graphs.

significant increases in both the average and maximum body surface temperature in lighter pups, whereas no such increases were apparent in heavier pups. Furthermore, the present results confirm and extend findings of previous studies in the European rabbit by showing that lighter pups had significantly fewer neighbors than pups of inter-mediate and heavier body mass relative to their litter siblings by an average of 0.25 and 0.45 individuals, respectively [cf. 30]. The fine-scale infrared thermographic analysis employed here in situ revealed that surface temperatures of lighter pups were on average 0.5 °C and 0.75 °C cooler than those of intermediate and heavier siblings, respectively.

These findings emphasize that the litter huddle in rabbits, and very possibly in other altricial litter-bearing mammals, is not a homogeneous unit but rather is made up of micro-niches consistently occupied by certain categories of littermates largely determined by their relative, within-litter body mass [24,27]. Furthermore, as reported previously, the occupation of such niches has notable consequences for individual differences among littermates in their early growth and survival [24,25,30]. Now, in an extension of these studies, the present findings suggest that lighter pups have more to gain from increasing their number of huddle partners by even just one or two additional pups than

their heavier littermates.

At least two different although complementary mechanisms might account for this. On the one hand, lighter pups have a less favorable body surface area-to-volume ratio than their heavier siblings and thus could be considered to be generally more vulnerable to challenging environmental conditions (e.g., [47] in juvenile wild rabbits shortly after weaning). Additionally, lighter rabbit pups have been shown to have significantly lower energy reserves in the form of brown adipose tissue [37]. On the other hand, as lighter pups generally have fewer neighbors than heavier ones and thus typically a comparatively smaller body surface in contact with littermates, they potentially have more to gain from increasing their number of neighbors. In support of this, changes in the number of litter neighbors had comparatively little effect on heavier siblings as these were typically already in body contact with comparatively more littermates (Fig. 2). Thus, the temporary addition (or loss) of one or two neighbors did not markedly alter their thermal niche. These findings are broadly consistent with a previous report that providing single rabbit pups with one littermate substantially increased their growth, survival and body temperature, that providing two lit-termates increased these benefits somewhat more, but that the benefits of increasing the number of littermates still further were marginal [6].

The present findings also help explain why lighter pups with lower energy reserves [37] invest more time and presumably energy than their heavier sibs in rooting between and climbing over littermates, apparently in an attempt to gain contact with at least one additional neighbor ([27,30]; see [29] for similar phenomena in laboratory rat pups). Physiologically, this means that lighter pups are burning more brown adipose tissue than their heavier littermates [36]. Behaviorally, it means that they are performing many more, apparently strenuous and presumably energetically costly goal-directed motor behaviors. And psychologically, despite the energetic costs for lighter pups of maintaining an adequate body temperature due to their poorly insulated, peripheral position in the litter huddle, that they must be strongly motivated to invest in doing so. Thus, taken together, the evidence from the present and previous studies for such dynamics within rabbit litters suggests that individual pups experience very different exogenous and endogenous early developmental contexts largely determined by their body mass relative to their littermates at birth [27,28,30]. This is in turn predicted by their intra-uterine position, which might be the main driver of variation in birth mass in the rabbit as well as in other polytocous mammals [48–51].

Finally and more speculatively, these results also have possible implications for the development of long-term individual differences in physiological and behavioral phenotypes among mammalian littermates, and even in genetically standardized laboratory animals that are often considered to be raised under identical conditions [52,53]. Although possible long-term behavioral consequences of such seemingly subtle differences in relative body mass and associated relations among litter siblings now need to be systematically tested, there is already evidence that this might be the case both in domestic rabbits [35] and in wild rabbits under semi-natural conditions [38].

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.physbeh.2017.06.005.

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VII. Capítulo 2: Sibling differences in litter huddle position contribute to overall variation in weaning mass in a small mammal

Considerando que las diferencias individuales en peso corporal modulan las diferencias individuales durante la termorregulación conductual (posición dentro del agrupamiento de la camada/*rooting y climbing*), y que son las crías más pesadas y centrales quienes obtienen más leche y la convierten con mayor eficiencia en biomasa, están más calientes, ganan más peso, y que al final de la primera semana posnatal persisten esas diferencias individuales (Rödel y cols. 2008), en nuestro segundo capítulo, mediante el uso de un análisis multivariado (*Path analysis*) evaluamos los efectos de las relaciones causales entre estas variables, ahora considerándolas como mecanismos que originan las diferencias individuales en crecimiento durante el desarrollo temprano. Las primeras semanas de vida son determinantes para dar forma a la estructura de la población a largo plazo en el conejo europeo. Esto debido a que la mortalidad durante los primeros 20 días de vida es cercana al 33 % (Rödel y cols. 2009), y solo cerca del 6 % de los individuos nacidos alcanzan a sobrevivir hasta su primer evento reproductivo (von Holst y cols. 1999). De este modo, en una aproximación a nivel poblacional evaluamos el efecto del peso al nacimiento, la competencia por sitios térmicamente ventajosos, y la reducción del tamaño de camada por muerte de las crías, sobre el crecimiento cuando los individuos están próximos a ser destetados.

El trabajo que a continuación se presenta está en preparación, los datos utilizados fueron obetnidos del año 2015 al 2018 en camadas del conejo doméstico. Los resultados mostraron que las interacciones entre hermanos en edades tempranas, específicamente durante el agrupamiento que forman para termorregular dentro del nido, dan forma a la variación intra-camada y poblacional en crecimiento en crías del conejo doméstico. Es decir, Son los individuos más pesados al interior de cada camada y a nivel poblacional quienes crecen más. Sin embargo, es mediante la conducta termorregulatoria intra-camada que se originan diferencias individuales en crecimiento a la par de el peso absoluto con el que nacen las crías.

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Sibling differences in litter huddle position contribute to overall variation in weaning mass in a small mammal
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| **Abstract:** | In altricial, litter-bearing species, huddling together with siblings during early life is a vital strategy to maintain a sufficiently high and stable body temperature. In this context, individual differences in huddling behavior within litters has been emphasized, as pups regularly occupying more central positions have relatively higher body temperatures, quicker access to the mother's nipples during nursing, and consequently show greater growth. However, it is not known whether such positive effects of a central litter huddle position on within-litter differences in growth translate into an overall higher weaning mass, taking into account strong contributors to among-litter |

growth variation such as litter size and maternal parity. We used path analysis to investigate causal relations among these variables, based on data from 150 domestic rabbit pups from 24 litters. Our results confirmed positive, indirect effects of pups' central litter huddle position on within-litter differences in early growth. This positive effect of a central litter huddle position also contributed to explaining a significant part of the overall across-litter variance in weaning body mass, apparent even when controlling for the direct negative effect of litter size, the direct positive effects of birth mass and the lower offspring growth in primiparous compared to multiparous mothers. Thus, the results underline the key role of individual differences in litter huddle position in shaping within-litter but also overall variation in early growth. This might constitute an important mechanism accounting for how the positive association between body mass at birth and early growth is mediated in altricial, polytocous mammals.

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1 # Sibling differences in litter huddle position contribute to

2 # overall variation in weaning mass in a small mammal

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1

17 **Abstract**

18 In altricial, litter-bearing species, huddling together with siblings during early life is a vital strategy

19 to maintain a sufficiently high and stable body temperature. In this context, individual differences in

20 huddling behavior within litters has been emphasized, as pups regularly occupying more central

21 positions have relatively higher body temperatures, quicker access to the mother's nipples during

22 nursing, and consequently show greater growth. However, it is not known whether such positive

23 effects of a central litter huddle position on within-litter differences in growth translate into an

24 overall higher weaning mass, taking into account strong contributors to among-litter growth

25 variation such as litter size and maternal parity. We used path analysis to investigate causal relations

26 among these variables, based on data from 150 domestic rabbit pups from 24 litters. Our results

27 confirmed positive, indirect effects of pups' central litter huddle position on within-litter differences

28 in early growth. This positive effect of a central litter huddle position also contributed to explaining

29 a significant part of the overall across-litter variance in weaning body mass, apparent even when

30 controlling for the direct negative effect of litter size, the direct positive effects of birth mass and

31 the lower offspring growth in primiparous compared to multiparous mothers. Thus, the results

32 underline the key role of individual differences in litter huddle position in shaping within-litter but

33 also overall variation in early growth. This might constitute an important mechanism accounting for

34 how the positive association between body mass at birth and early growth is mediated in altricial,

35 polytocous mammals.

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39

40   **Significance statement**

41   Huddling together with siblings during early life saves energy and thus can contribute to early

42   growth in small, altricial mammals. But this strategy can also lead to individual differences within

43   the litter, as heavier pups typically occupy energetically more favorable positions in the center of

44   the huddle. In our study we show and compare the different causal pathways underlying this effect.

45   Most importantly, our analysis shows that advantages in early growth arising from a more central

46   position in the litter huddle are also apparent when comparing pups across all litters – even though

47   there is typically a notable variation in growth among different litters, for example due to litter size

48   and maternal (parity) effects. In conclusion, the results underline the key role of sibling interactions

49   within the litter in shaping differences in early growth, with potential fitness consequences during

50   later life.

51

Introduction

Growth during early postnatal life is a key trait characterizing variation in individual life histories. Animals with comparatively lower growth rates and resulting body masses frequently suffer from negative fitness consequences (Lindström 1999; Metcalfe and Monaghan 2001; Lummaa and Clutton-Brock 2002; Ronget et al. 2018). For example in mammals, early growth and body mass at around weaning have been shown to be positively associated with an animal's survival during early life stages (Lenihan and Van Vuren 1996; Marboutin and Hansen 1998; Kraus et al. 2005; Monclús et al. 2014), its breeding success (Haywood and Perrins 1992; Festa-Bianchet et al. 2000), and its competitive strength during adulthood (Rödel and von Holst 2009).

Various environmental factors and individual-based characteristics including their interplay have the potential to shape individual differences in early growth. In particular in animals with a higher degree of parental care, quality characteristics of the caregiver(s) are decisive (Lim et al. 2014). In mammals, maternal body condition (Dobson and Michener 1995; Skibiel et al. 2009) and age (Derocher and Stirling 1998; Rödel et al. 2008c), as well mother's parity (Côté and Festa-Bianchet 2001; Altmann and Alberts 2005) can affect offspring growth. Furthermore, across a wide range of taxa, the number of offspring is negatively related with offspring size (Lim et al. 2014). In polytocous mammals, offspring from larger litters typically obtain a lower share of maternal resources (mostly milk) thus leading to lower birth masses, lower postnatal growth rates and resulting weaning masses compared to offspring with a smaller number of littermates (Mendl 1988; Rödel et al. 2008c). As a consequence, siblings might benefit from their littermates' death – which frequently occurs in small altricial mammals with typically high nest mortalities (Millar 2007; Rödel et al. 2009) – as this will increase their individual share of milk (Drummond et al. 2000). However, having a higher number of siblings does not only impose costs to the individual offspring. During the first postnatal days, when altricial mammals still have an incomplete ability to

76 thermoregulate independently (Hull 1973; Zepeda et al. 2018), huddling together with a higher

77 number of littermates provides thermal and energetic benefits, improving their growth (Bautista et

78 al. 2003; Gilbert et al. 2007; Rödel et al. 2008b; Gilbert et al. 2010; Harshaw and Alberts 2012).

79   Also within a litter, there are notable differences in early body mass and associated

80 differences in individual growth trajectories. Individuals born with a lower body mass - often the

81 consequence of their relative position in utero, that is, the site of implantation of a fetus relative to

82 its littermates (Kinsley et al. 1986; Ryan and Vandenbergh 2002; Bautista et al. 2015a) – typically

83 show lower growth than their heavier littermates (Rödel et al. 2008a). The mechanisms underlying

84 such within-litter differences are particularly well studied in the domestic rabbit (*Oryctolagus*

85 *cuniculus*). Rabbit pups, born naked and with poor thermal insulation, huddle together immediately

86 after birth so as to maintain a sufficiently high and stable temperature in the litter huddle (Hull and

87 Hull 1982). Relatively heavier siblings, who already have a slightly higher body temperature shortly

88 after birth due to their more favorable body-surface-to-volume ratio, quickly occupy more central

89 positions in the litter huddle (Bautista et al. 2015b). Recent studies indicate that by occupying these

90 typically warmer central positions, heavier pups can efficiently save energy by lower investment in

91 thermoregulation (García-Torres et al. 2015), and are able to obtain more milk during nursing and

92 to convert it more efficiently into biomass,, resulting in better growth compared to their lighter

93 siblings occupying more peripheral positions (Bautista et al. 2005; Rödel et al. 2008a; Bautista et al.

94 2015b). However, even though there is a certain predisposition in the siblings' litter huddle position

95 due to their relative body mass, studies in domestic rabbits as well as in other altricial small

96 mammals show that individuals typically attempt to actively improve their position by directed

97 locomotor behavior (climbing over and rooting under and between their littermates) towards the

98 thermally more advantageous center (Alberts 1978; Bautista et al. 2008; Bautista et al. 2010;

99 Zepeda et al. 2018). In summary, the European rabbit provides a striking example of how

100   differences in micro-niches within the litter huddle can directly affect growth of the young and lead

101   to the maintenance of individual differences in developmental trajectories among littermates (Rödel

102   et al. 2008a). It however remains open whether and to what extent such sibling interactions

103   contribute significantly to overall individual variation in growth even across different litters. This

104   would imply that the outcome of individual growth differences related to the position in the litter

105   huddle will be apparent at the population level despite the usually large between-litter variation in

106   offspring growth, for example because of differences in litter size and in maternal characteristics

107   (Mendl 1988; Rödel et al. 2008c).

108   We carried out our study in a breeding facility of domestic rabbits over 4 years, using a total

109   of 24 litters all from different females. In a first step, we (*i*) summarize the mechanisms, which have

110   been previously described in different studies (reviewed in: Rödel et al. 2008a; Hudson et al. 2011)

111   by exploring the purported chain of causes and consequences using multilevel path analysis

112   (Shipley 2009). Based on our previous studies, we predicted that litter-sibling (relative) differences

113   in body mass should contribute notably to explaining relative differences in early growth, mainly

114   via advantages related to more central position in the litter huddle (Bautista et al. 2015b). Most

115   importantly, we (*ii*) explored whether individual differences in litter huddle position translated into

116   an overall higher individual weaning body mass. We predict that individual differences in the

117   position in the litter huddle will still make a significant contribution to the observed differences in

118   weaning body mass when taking into account other well-documented sources of variation shaping

119   between-litter differences in growth, such as litter size at birth (Poigner et al. 2000; Rödel et al.

120   2008c), litter size reduction during the first postnatal week (Drummond et al. 2000) and the parity

121   (primiparity vs. multiparity) of the mother (Prayaga and Eady 2003).

122

123

Methods

124

125 **Animals and housing conditions**

126 We collected the data between September 2014 and August 2018, initially from 193 pups of 24 un-

127 culled litters. However, the sample size decreased to $N = 150$ pups (76 females and 74 males) when

128 they were 10 days old and by the end of the study (day 25) due to pup mortality (2 female and 1 male)

129 to $N= 147$. The litters came from 24 different (4 primiparous and 20 multiparous) mothers each mated

130 with one of 16 different stud males, bred and maintained at the Centro Tlaxcala de Biología de la

131 Conducta, Mexico. Females were kept in individual stainless steel cages $90 \times 60 \times 40$ cm high and

132 under fluorescent lights set to a 16:8 h light:dark cycle, which approximates conditions at the height

133 of the summer breeding season for rabbits in Europe. Ambient air temperature was maintained

134 between 17 and 24 °C, and water and food (Purina rabbit chow, Purina®, USA) were continuously

135 available. On day 28 of gestation, around three days before parturition, mothers were given hay and

136 an open-top wooden box ($40 \times 35 \times 15$ cm high) in which to build a nest. At the end of the experiment

137 (postnatal day 25) the pups were sexed by visual inspection or in the case of early death, they were

138 sexed surgically.

139   Mean litter size at birth was 8.0 (min = 6 and max = 11). A total of 43 pups (22.3%) died in

140 the 24 litters. The majority of deaths (37 out of 43; 86.0%) occurred during the first postnatal week.

141 We considered the effects of litter size through this critical developmental period, taking into

142 account daily mortality from postnatal day 0 to 7 when the huddling experiment was concluded (see

143 below), and calculating proportional decreases in litter size (range 0 to 55.6%).

144

145 **Experimental procedure**

146 Experiments (2014-2018) were carried out by different graduate students, although all were trained

147 and supervised by the same senior researcher (AB).

148

Individual marking of pups

On the day of birth (postnatal day 0), we removed the nest boxes from the mothers' cages, we

weighed the pups on an electronic balance (Ohaus, Navigator) to the nearest 0.1 g, and in random

order each pup (dark gray in this breed) was numbered on the back, flanks, and ventrum with white

correcting fluid (Nukote, Pelikan) for individual identification in the video recordings (see below).

The litters were then returned to their mother's cage in their nest boxes until the following day.

Individual differences in pups' position in the litter huddle

During postnatal days 1–7, the period when pups have little fur and are critically dependent on body

contact with littermates to maintain an adequate and stable body temperature (Hull and Hull 1982;

Bautista et al. 2003; Gilbert et al. 2007), we quantified individual within-litter differences in

position within the huddle by recording the number of neighbors with which each pup was in body

contact. For this, on postnatal day 1 at 09:00 h we weighted them and then placed them in a flannel-

lined nest box but without nest material to allow behavioral recording. To prevent pups becoming

trapped in the corners they we placed inside a 28-cm diameter wire mesh hoop. The box was placed

in continuous light in a cool room and remained there until postnatal day 7. To induce pups to

huddle the temperature of the room was first set at 25 °C, and thus below the thermo-neutral zone

for new-born rabbits of about 35 °C (Hull 1965). This induces huddling behavior, but is not so low

as to threaten pups' survival (Bautista et al. 2003; cf. Rödel et al. 2008b for soil temperature

conditions around nursery burrows in wild rabbits). To ensure the thermal importance of the huddle

despite pups' increasing body volume and growth of fur, the ambient temperature was lowered by 1

°C each day after the last daily measurement of body temperature (see below). An acrylic cover was

placed over the box to protect the pups from air currents, and a video camera was mounted above to

172   record their behavior. Litters were filmed for 10, 15-min sessions during each 24 h from postnatal

173   days 1 to 7 (at 07:00, 09:00, 12:00, 14:00, 16:00, 18:00, 23:00, 01:00, 03:00, 05:00 h).

174   To quantify pups' number of neighbors in the huddle, we analyzed four frames from each

175   video – at 0, 5, 10 and 15 min – giving a total of 40 frames per day/litter. For each pup in each

176   frame we counted the number of littermates (neighbors) with which it was in contact with an

177   estimated 10% or more of its body surface. From this, we obtained the mean number of neighbors

178   with which each pup was in contact during the first seven postnatal days (Reyes-Meza et al. 2011;

179   Bautista et al. 2015b).

180

181   *Peripheral body temperature*

182   Given the altricial condition of the pups at this age and the co-dependency of thermoregulation and

183   growth (Gilbert et al. 2007; Gilbert et al. 2010), we also measured individual differences in

184   peripheral body temperature. For this, we measured each pup's skin temperature five times during

185   each 24 h (at 9:00, 11:00, 13:00, 15:00, 17:00 h) at the throat and groin using a digital thermometer

186   (Thermocouple Fluke® 52 II), from postnatal days 2 to 7. These temperature values were averaged

187   for each pup for later analysis.

188

189   *Milk intake and milk conversion index*

190   The pups were nursed for approximately 3 min each day as is normal in the rabbit (Deutsch 1957;

191   Zarrow et al. 1965). To measure individuals' milk intake, before pups were nursed we induced them

192   to urinate by applying gentle pressure to their bladder from postnatal day 2 to 6 at 10:00 h.

193   Urination was induced to enable accurate measurement after nursing of milk intake. Pups were then

194   individually weighed and returned to their nest box after removing the mesh hoop to enable mothers

195   to adopt a normal nursing posture over the pups. At the end of the nursing event, indicated by the

196 mother jumping out of the box, we again weighed the pups and took the difference between their

197 pre- and post-nursing weight as a measure of their milk intake. Then, as a measure of pups'

198 efficiency in converting milk into body mass we calculated an index by dividing the total body mass

199 increase from postnatal day 2 to 7 (measured shortly before nursing) by the summed daily milk

200 intake (see above) from postnatal days 2 to 6. (Drummond et al. 2000; Bautista et al. 2003).

201

202 *Pre-weaning growth*

203 By 7 days of age the pups have a good cover of fur and considerably improved motor control

204 (Muciño et al. 2009), and by postnatal day 10 they have usually opened their eyes, started mouthing

205 and nibbling the nest material, and start exploring their surroundings (Zarrow et al. 1965; Hudson

206 and Distel 1982; Hudson et al. 1996). To investigate individual differences in growth during this

207 early developmental period when mortality is high and negatively related to pups' body mass

208 (Rödel et al. 2009), we used the increase in body mass from birth to postnatal day 10 as a first

209 measure of early growth. For this, we subtracted the body mass at birth from the body mass on

210 postnatal day 10. In addition, we also weighed the pups on day 25, just before they were

211 permanently separated from their mother at weaning, as body mass at around weaning is considered

212 a good predictor of different life history parameters across a wide range of taxa in mammals and in

213 birds (Ronget et al. 2018).

214

215 **Statistical analysis**

216 To minimize observer bias, all behavioral and physiological data from the several previous studies

217 combined for the path analysis of the present report were recorded and analyzed using blinded

218 methods.

219   Analyses were done with the software R version 3.5.3 (R Core Team 2018). We calculated

220   the causal relationship among variables using path analysis with the R package *piecewiseSEM*

221   (Lefcheck 2016), based on linear mixed-effects models calculated with the R package *lme4* (Bates

222   et al. 2015). We started with a direct acyclic graph (DAG) to explore the causal relationship

223   between relative body mass at birth (within-litter variation, calculated as the deviation from the

224   litter mean), sex, the relative number of neighbors in the litter huddle, the relative milk intake,

225   relative skin temperature, relative milk conversion, and relative growth at postnatal day 10 (see Fig.

226   1a). We applied d-separation to the set of independence claims, and for that, we fitted linear mixed-

227   effects models with a Gaussian distribution, and with litter identity as a random factor (Pearl 2009;

228   Shipley 2009). We tested the goodness of fit of the final DAG by a Fisher's C test (Shipley 2009).

229   For path orientation, we used the temporal tiers (events that happen earlier in time can only be

230   parental nodes of variables that happen later in time). To orient the variables within the same tier we

231   calculated the dependencies and independencies of triplets of variables to exclude colliders

232   (Scheines et al. 1994). Relative milk intake and relative differences in peripheral body temperature

233   were strongly and positively correlated ($R^2 = 0.778$, $n = 150$, $P < 0.001$), and thus we computed two

234   alternative paths, each with just one of the correlated variables.

235   We also built a DAG for the overall variation in body mass at weaning, also using linear

236   mixed-effects models with a Gaussian distribution, and with litter identity as a random factor. The

237   nodes of the DAG were body mass at birth, litter size at birth, the number of neighbors in the litter

238   huddle, the % decrease in litter size from day 0 to 7 (and thus a decrease in the number of potential

239   neighbors but also in competitors for milk), mother's parity (primiparous vs. multiparous), and body

240   mass at postnatal day 25 (see Fig. 1b).

241   Analysis revealed no significant paths between sex and any of the variables considered (all

242    $P > 0.10$). Thus, sex is not depicted in any of the final DGAs (Fig. 1a, b).

243   For analysis, data were standardized by scaling them, i.e. we subtracted the mean from each

244   variable and divided it by the standard deviation. This allowed us to obtain standardized slopes (β),

245   and the absolute value of the standardized slope (beta weight) can be interpreted as the relative

246   effect size of an association (see Fig. 1a, b). The support of a path in comparison with an alternative

247   path can be calculated by the multiplication of the different slopes of this path (Lefcheck 2016).

248   Each of the linear mixed-effects models used for the calculation of the paths was checked for

249   normal distribution of residuals by normal probability plots and we verified homogeneity of

250   variances by plotting residuals versus fitted values (Faraway 2006).

251   We also present marginal pseudoR^2 for each correlation included in the path diagram (Fig.

252   1), which can be interpreted as the proportional explained variance excluding the contribution of the

253   random factor to the explained variance (Nakagawa and Schielzeth 2013).

254

Results

Paths explaining within-litter variation in early growth

257   The model outlined in Fig. 1a was an adequate fit to the data as verified by a goodness of fit test

258   (Fisher's $C = 7.238$, $P = 0.299$). All variables included in this path analysis are relative values

259   calculated as the % deviation from the litter mean, reflecting differences among litter siblings.

260   There were no significant associations of sex with any of the variables included in the analysis (all

261    $P > 0.10$).

262   Relative body mass at birth was positively and significantly associated to relative growth

263   (from postnatal day 0 to day 10) by different indirect paths via the relative number of huddling

264   partners (thus reflecting position within the litter huddle), via relative (peripheral) body

265   temperature, and relative milk intake (Fig. 1a). The total effect of mass at birth on growth by day 10

266   via these two paths was 0.604 (standardized β: [$0.640 \times 0.634 \times 0.772$] + [$0.640 \times 0.729 \times 0.623$]).

267   The indirect effect of relative position in the litter huddle on early growth via relative milk

268   intake ($0.634 \times 0.772 = 0.489$) and the indirect effect of relative huddling position on early growth

269   via relative differences in body temperature ($0.729 \times 0.623 = 0.454$) were very similar in

270   magnitude. The model predicted further significant, indirect paths according to which the relative

271   position in the huddle affected the relative growth via the effects on body temperature on milk

272   intake ($0.729 \times 0.740 \times 0.772 = 0.416$), or vice versa ($0.634 \times 0.740 \times 0.623 = 0.293$). The direction

273   of the association between milk intake and peripheral body temperature could not be disentangled

274   by conditional independence claims (Fig. 1a), and both directions of this association find reasonable

275   biological support.

276   We also found support for a direct positive path between relative body mass at birth and

277   relative milk intake, and surprisingly we found a direct negative path between relative birth mass

278   and the relative milk conversion rate.

279   The model did not support any direct significant effect of our measure of relative milk

280   conversion on the relative increase in body mass (Fig. 1a).

281

282   --- Fig. 1 about here ---

283

284   **Paths explaining the overall variation in body mass around weaning**

285   Also here, a goodness of fit test confirmed that the model outlined in Fig. 1b was an adequate fit to

286   the data (Fisher's $C = 0$, $P = 1$). And again, there were no significant associations of sex with any of

287   the variables considered (all $P > 0.10$).

288   The overall variation in weaning body mass within and across litters was significantly

289   explained by the additive effects of 5 of the predictor variables considered (Fig. 1b); that is, body

290   mass at birth, the % decrease in litter size during the early postnatal period, and position in the litter

291   huddle as assessed by the average number of neighbors a pup was in contact with (see details of this

292   effect in Fig. 2) were significantly and positively correlated with weaning mass, whereas litter size

293   was negatively correlated with weaning body mass. Furthermore, offspring from multiparous

294   mothers grew better than offspring from primiparous ones.

295   Notably, body mass at birth, litter size, and % decrease in litter size showed direct paths to

296   weaning body mass (standardized β: 0.209, –0.521, and 0.350, respectively) as well as indirect

297   paths to weaning mass via position in the litter huddle. These indirect paths to weaning mass

298   showed lower magnitudes than the direct paths (body mass at birth via position in litter huddle:

299      $0.556 \times 0.425 = 0.236$; litter size via position in litter huddle: $0.580 \times 0.425 = 0.247$; % decrease in

300   litter size via position in litter huddle: $–0.351 \times 0.425 = –0.149$).

301

302   --- Fig. 2 about here ---

303

304   Discussion

305   Pups who were in body contact with a larger number of siblings in the litter huddle during the first

306   postnatal days showed relatively higher growth than littermates with less body contact. According

307   to our hypothesis, this effect also contributed significantly in explaining the overall variation in

308   weaning mass in our sample of 150 juveniles from 24 litters. The present study extends the results

309   of previous experimental work in the domestic rabbit (e.g., Bautista et al. 2008; Rödel et al. 2008a;

310   Bautista et al. 2015b) by using path analysis to examine the direction and relative contribution of

311   various factors to individuals' early growth and weaning mass.

312   In a first step (Fig. 1a) we used data for litters from birth to postnatal day 10, and focused

313   on relative differences among littermates. We limited the analysis to day 10 because this is the

314   period of highest nest mortality in the European rabbit (domestic rabbit: Coureaud et al. 2000;

315   Drummond et al. 2000; wild rabbit: Rödel et al. 2009), and because by this age the altricial pups

316   start to open their eyes, have a good coat of insulating fur, improved motor skills and start to eat

317   material from the nest constructed by the mother (Hudson et al. 1996). In addition, sibling

318   interactions, in terms of both costs and benefits, have been repeatedly shown to be important

319   shapers of survival and growth during this first critical developmental bottleneck (Mock and Parker

320   1997; Bautista et al. 2003; Gilbert et al. 2007; Rödel et al. 2008b). The path analysis performed here

321   basically confirms our previous findings but also provides additional information on the

322   directionality and relative strength of the various factors entered into the model. From this, it is

323   clear that the strong influence of differences in body mass at birth among litter siblings on the

324   relative differences in their body mass on day 10 is mainly driven by pups' relative position within

325   the litter huddle via the effect of this on within-litter differences in milk intake and body

326   temperature. Pups who are relatively heavier at birth maintain body contact with a larger number of

327   neighbors, i.e., occupy more central positions within the huddle (Bautista et al. 2015; see similar

328   effects in the laboratory rat *Rattus norvegicus*: Bautista et al. 2010; and in the house mouse *Mus*

329   *musculus*: Zepeda et al. 2018). As a consequence, in a positive feedback loop, heavier, more central

330   pups have a relatively higher milk intake and relatively higher body temperature than their lighter

331   siblings in body contact with fewer neighbors and occupying more peripheral positions in the

332   huddle (Bautista et al. 2008; Rödel et al. 2008a; Bautista et al. 2015b). This results in them having a

333   greater body mass on day 10.

334   These new results reinforce previous findings pointing to the importance of sibling

335   interactions in shaping individual differences in early weight gain (growth) in this altricial mammal

336   (Rödel et al. 2008a). Although it is well-known, and indeed might seem obvious that early growth

337   would be positively influenced by milk intake and good insulation within the litter huddle, the

338   potential role of siblings as mediators of this process is less often considered. And yet such a pattern

339 is likely to be similar for other altricial, polytocous species also. Although it might be argued that

340 such a pattern might be specific to the European rabbit given this species' system of "absentee"

341 maternal care (see Broekhuizen et al. 1986; Rödel et al. 2012 for confirmation under natural or

342 quasi-conditions), this might not be as unusual or extreme as it first appears. Studies based on

343 laboratory or captive animals may overestimate the time mothers spend with their young, both

344 because of limited cage space and lack of opportunity for mothers to distance themselves from their

345 young, and the often ad libitum provision of food and water. Under natural conditions mothers need

346 to leave their young for considerable periods to forage while at the same time needing to avoid

347 being preyed upon (Monclús and Rödel 2009) and being possibly involved in competitive social

348 interactions with other females of the population (Rödel et al. 2008d; Seltmann et al. 2017).

349 Foraging demands are particularly high during lactation when food intake and feeding time

350 increases greatly to meet the high energetic costs of milk production (Speakman 2008; domestic and

351 wild rabbit: Martínez-Gómez et al. 2004; Rödel et al. 2016).

352   However, while the pathways shown in Fig. 1a confirm and reinforce the contribution of

353 sibling interactions to the early development of individual differences in body mass in the rabbit,

354 this analysis refers to relative differences among individuals within litters. To know whether such

355 differences are of actual ecological relevance, it becomes necessary to ask whether they also apply

356 to differences in body mass at weaning at the population level and so to an environment in which

357 the young from across litters will be confronted when they leave the nursery burrow. A first

358 examination of this question was the purpose of the path analysis shown in Fig. 1b. Specifically, we

359 asked whether the effect on individual differences in body mass of sibling interactions within the

360 nest up to day 10 would persist to weaning, taking into account the body mass of all young from all

361 litters, that is, of the whole population comprising the present study.

362   Results of the path analysis in Fig. 1b show that four variables had a direct effect on

363   individuals' body mass at weaning, independently of pups' relative positions in the litter huddle.

364   These four variables were mothers' parity, litter size at birth, decrease in litter size and to a lesser

365   extent, pups' body mass at birth. These effects are not particularly surprising. Returning to the

366   effect of parity, it is widely recognized that in iteroparous mammals and birds, reproductive

367   performance as measured by litter or clutch size, milk production or feeding effort, and other

368   aspects of maternal care such as nest building improve after the first reproductive event, with

369   positive consequences for the body mass of the young at weaning or fledging (Sæther 1990; Côté

370   and Festa-Bianchet 2001; Rödel et al. 2004; Altmann and Alberts 2005; Hudson et al. 2011). Litter

371   size is also well known to affect weaning body mass as the young of larger litters generally obtain

372   less milk than young from smaller litters (different small mammals: Mendl 1988; Rödel et al.

373   2008c). For a similar reason, a decrease in litter size due to pup mortality (or culling), has a positive

374   effect on weaning mass as the remaining littermates then obtain a greater share of the mother's milk

375   (Drummond et al. 2000). Our present analysis also confirms the often reported finding that the

376   young from smaller litters are generally heavier at birth (deer mouse *Peromyscus maniculatus*:

377   Myers and Master 1983; red squirrel *Sciurus vulgaris*: Wauters et al. 1993; greater guinea pig *Cavia*

378   *magna*: Kraus et al. 2005; yellow-bellied marmot *Marmota flaviventris*: Monclús et al. 2014),

379   which in turn showed a direct positive effect on body mass at weaning in our study.

380   Most importantly, despite these multiple direct effects on individual differences in weaning

381   weight, pups' relative position within their respective litter huddles continued to have an effect as

382   well. Echoing the results shown in Fig. 1a, pups with a greater body mass at birth maintained body

383   contact with a larger number of neighbors in the huddle, and this had an overall positive effect on

384   their body mass at weaning at the population level (Fig. 2). In fact, this effect was even greater than

385   for the other variables contributing directly to weaning mass except for the slightly stronger

386   influence of litter size at birth. In other words, according to the two path analyses of the present

387   study, interactions among siblings, as measured by their relative positions in the litter huddle during

388   early postnatal development, remains a significant contributor to their body mass at weaning even

389   when calculated at the level of the general population, meaning that this usually little-considered

390   variable may indeed be of ecological significance.

391   Here, however, the criticism can be made that the analyses of the present study were based

392   on litters from laboratory animals of a particular genetic stock and kept in conditions very different

393   to those of wild populations. For example, not included in the models are variables likely to affect

394   offspring body mass and early growth in nature such as for example prevailing weather conditions

395   (Rödel et al. 2008b), maternal body condition or body mass (Dobson and Michener 1995; Skibiel et

396   al. 2009; Rödel et al. 2016), and parasitic load and disease (Reed et al. 2012), which might possibly

397   result in a more complex pattern of predictor pathways and their relative strength. Nevertheless,

398   where comparable studies in European rabbits under natural conditions exist they have shown

399   results consistent with those reported here for domestic rabbits and as shown by the present path

400   analysis to be important for individual differences in early postnatal (Fig. 1a) and weaning body

401   mass (Fig. 1b). For example, a study in a field enclosure under quasi-natural conditions showed

402   lower pre-weaning growth in offspring from primiparous females together with strong negative

403   litter size effects on birth mass and pre-weaning growth (von Holst et al. 2002; Rödel et al. 2008c),

404   consistently lower growth in pups with relatively lower birth mass (Rödel et al. 2008a), and higher

405   body mass gain in pups which were relatively warmer than their littermates during the first

406   postnatal days (Rödel et al. 2017). Furthermore, studies in the European rabbit have shown that high

407   juvenile body mass before leaving the nursery burrow at around postnatal day 20 can contribute

408   importantly to an individual's chances of survival during the following weeks (Rödel et al. 2015).

409   Finally, our study constitutes an important starting point to examine the longer-term consequences

410 of early life conditions in polytocous animals, stressing the importance of including sibling effects

411 during early life as modulators of life-history trajectories.

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433

434   **Conflict of interest** The authors declare they have no conflict of interest.

435

436   **Data availability** The authors confirm that the data supporting the study will be made available by

437   the corresponding author upon reasonable request.

438

439   **Ethics statement** All applicable international, national, and institutional guidelines for the care and

440   use of animals were followed. All procedures performed were in accordance with the ethical

441   standards of the institution at which the studies were conducted, and were approved by the Animal

442   Care and Use Committee of the Centro Tlaxcala de Biología de la Conducta, protocol 056-2014-

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618

Figure legend

Fig. 1 Results of path analysis showing the strength of association between the variables considered

during the preweaning period (a) within-litter associations to postnatal day 10, and (b) across the

different litters at weaning on postnatal day 25. Arrows indicating positive associations are shown

by +, negative associations by –, and the relative strength of the association is indicated by the

absolute value of the beta weight and visualization by the thickness of the arrow. Only significant

paths ($P < 0.050$) are given; see text for details on statistics. Analyses were based (a) on 150 pups

and (b) 147 pups from 24 litters. The (marginal) pseudoR^2 for each correlation included in the path

diagram is given.

Fig. 2 Significant correlation between the average number of neighbors each pup was in contact

with during postnatal days 1-7 and body mass around weaning, measured on postnatal day 25

Statistics using a multifactorial linear mixed-effects model with litter identity as a random factor

($F_{1,140} = 29.010$, $N = 143$, $P < 0.001$); see other significant explanatory variables in Fig. 1b.

Figures
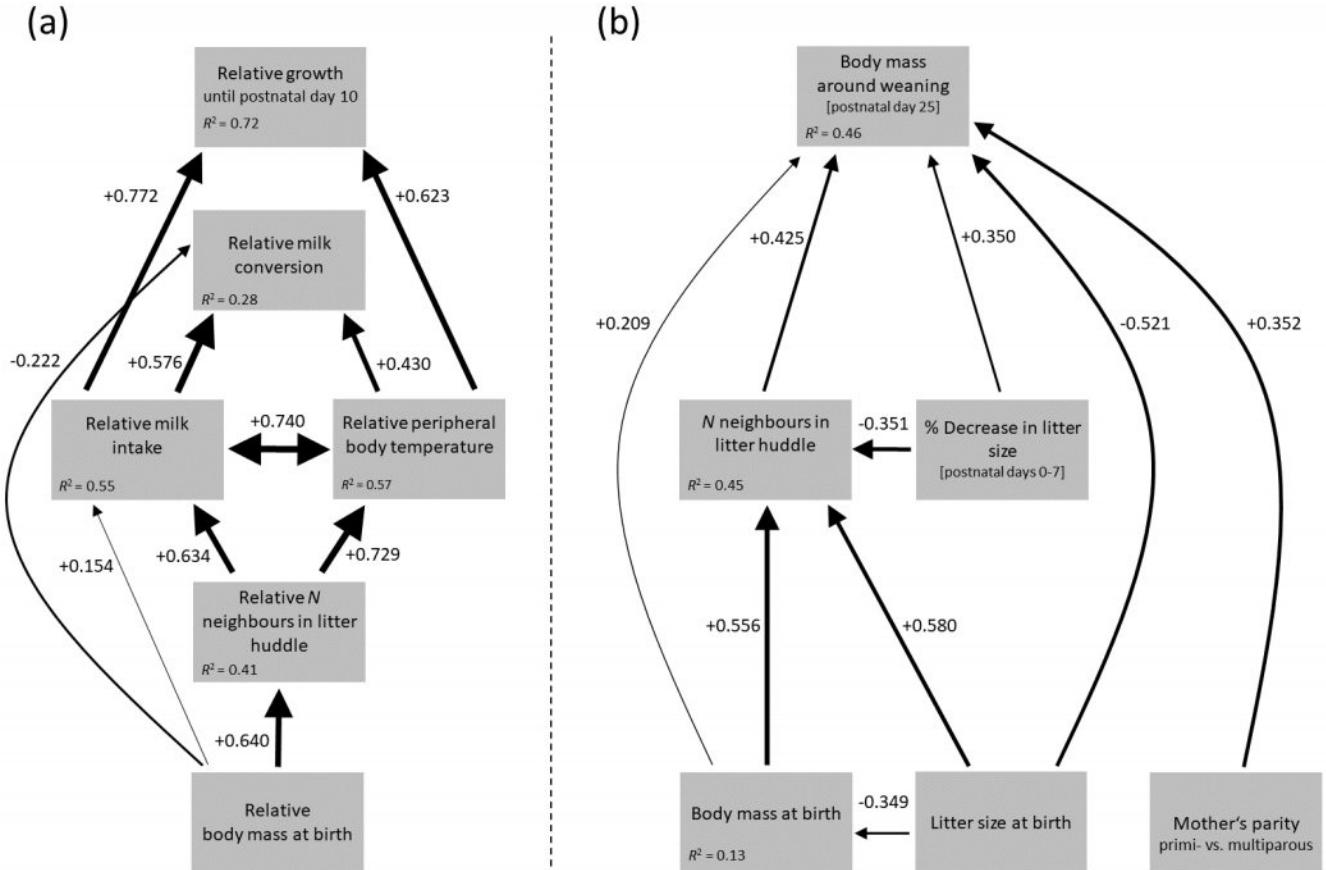
Fig. 1 Zepeda et al.

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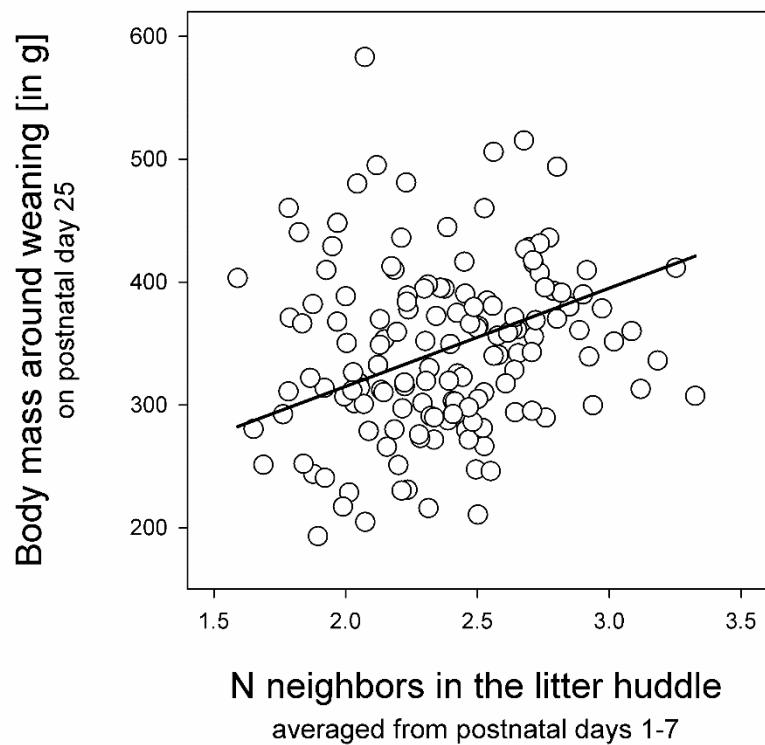
Fig. 2 Zepeda et al.

VIII. Capítulo 3: Individual differences in early body mass affect thermogenic performance and sibling interactions in litter huddles of the house mouse.

El agrupamiento como conducta termorregulatoria ya ha sido ampliamente explicado en crías de rata (Alberts, 1978a, 1978b), conejo (Bautista y cols. 2003, 2008) y ratón (Harshaw y Alberts, 2012). Sin embargo, hasta ahora no se ha evaluado el efecto de las diferencias inviduales en fisiología y conducta presentes en la vida temprana sobre el desarrollo la termorregulación en el ratón doméstico. Por esta razón, nuestro tercer capítulo consistió en evaluar en este roedor, ¿si la variación intra-camada en peso se asocian a diferencias individuales en el desempeño termogénico cuando son expuestos a un reto térmico, y si esta variación predice la competencia por acceder a los sitios térmicos más ventajosos del agrupamiento mediante conductas dirigidas para acceder al centro del agrupamiento (*rooting/climbing*)? Previamente hemos reportado en conejo y en rata (Bautista y cols. 2008, 2009) que son los individuos más ligeros de la camada quienes realizan estas conductas con mayor frecuencia. Además, nuestros resultados para este objetivo aportan evidencia para establecer hipótesis sobre relaciones evolutivas sobre los mecanismos que modulan la ontogenia de las diferencias individuales en edades tempranas y sus efectos a corto plazo.

El trabajo que a continuación se presenta, se publicó a partir de los datos generados en el laboratorio de etología evolutiva y comparada de la Universidad Sorbona de Paris, Paris XIII. Se utilizarón camadas de *Mus musculus* provenientes de parejas reproductivas capturadas en la vida silvestre, se registro continuamente sus capacidades termorregulatorias, su comportamiento dentro del agrupamiento intra-camada y se asocio a diferencias inividuales en el peso corporal inicial. Encontramos concordancia con lo que habiamos reportado previamente en ratas y conejos aquellos inividuos más ligeros defienden con menos exito su tempertatura corporal cuando son expuestos a un reto termico, además la variación intra-camada en el peso corporal inicial se asocian de forma negativa al número de desplazamientos realizados para acceder al centro del agrupamiento.

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WILEY Developmental Psychobiology

Individual differences in early body mass affect thermogenic performance and sibling interactions in litter huddles of the house mouse

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Abstract

We asked whether within-litter differences in early body mass are associated with differences in house mouse pups' thermogenic performance and whether such variation predicts individual differences in competitive interactions for thermally more advantageous positions in the huddle. We explored pups' thermogenic performance in isolation by measuring changes in (maximal) peripheral body temperatures during a 5-min thermal challenge using infrared thermography. Changes in peripheral body temperature were significantly explained by individual differences in body mass within a litter; relatively lighter individuals showed an overall quicker temperature decrease leading to lower body temperatures toward the end of the thermal challenge compared to heavier littermates. Within the litter huddle, relatively lighter pups with a lower thermogenic performance showed consistently more rooting and climbing behavior, apparently to reach the thermally advantageous center of the huddle. This suggests that within-litter variation in starting body mass affects the pups' thermal and behavioral responses to environmental challenges.

KEYWORDS
body temperature, early development, early life conditions, infrared thermography, *Mus musculus domesticus*

1 | INTRODUCTION

Mammalian species frequently show notable interindividual differences in birth mass (Bowen, 2009; Lummaa & Clutton-Brock, 2002; Milligan, Fraser, & Kramer, 2002). Variation in this trait can considerably affect an animal's subsequent growth and development, with consequences for survival and reproduction (Albon, Clutton-Brock, & Guinness, 1987; Lindström, 1999; Quiniou, Dagorn, & Gaudré, 2002). Typically, offspring born with smaller size and with a lower postnatal growth often suffer from a higher mortality prior to weaning and beyond (Côté & Festa-Bianchet, 2001; Guinness, Clutton-Brock, & Albon, 1978; Magnabosco, Bernardi, Went, Cunha, & Bortolozzo, 2016; Maniscalco, 2014; Rödel et al., 2015; Rödel, Starkloff, Seltmann, Prager, & von Holst, 2009).

In polytocous species, notable differences in early body mass can be often found within a litter (Hudson, Bautista, Reyes-Meza, Morales Montor, & Rödel, 2011; Milligan et al., 2002; Mock & Parker, 1997; Rödel, Bautista, García-Torres, Martínez-Gómez, & Hudson, 2008). Studies in different mammalian species, including the laboratory mouse *Mus musculus* indicate that such within-litter variation is mainly a consequence of the relative position of an individual in utero, that is, the site of implantation of a fetus relative to its littermates (Bautista, Rödel, et al., 2015; Kinsley et al., 1986; Ryan & Vandenbergh, 2002). In addition, such relative differences in starting mass among littermates can result in consistent sibling differences in growth and in resulting body mass at least across juvenile life stages (e.g., domestic and wild rabbit *Oryctolagus cuniculus*: Rödel, Bautista, et al., 2008). Sibling differences in early body mass can lead to individual differences in the way pups behave within the litter huddle (Hudson et al., 2011). Huddling together with litter siblings during early postnatal life is a highly efficient strategy of communal thermoregulation in altricial and polytocous species, helping to conserve energy essential for early growth and survival, and particularly in the absence of the mother or other care givers (Blumberg & Sokoloff, 1998; Gilbert et al., 2010). This has been intensively studied in different species of altricial mammals and birds (Alberts, 1978; Blumberg & Sokoloff, 1998; Forbes, 2005; Gilbert, McCafferty, Giroud, Ancel, & Blanc, 2012; Hill, 1983; Rödel, Hudson, & von Holst, 2008; Sokoloff & Blumberg, 2001), including the laboratory mouse (*Mus musculus*: Harshaw & Alberts, 2012; Harshaw, Culligan, & Alberts, 2014; Shelton & Alberts, 2018). Studies in the domestic rabbit and in the laboratory rat *Rattus norvegicus* have shown that pups with relatively lower birth mass typically occupy more peripheral positions in the huddle than their heavier siblings, despite showing comparatively more attempts to reach a thermally more advantageous position in the center (Bautista, García-Torres, Martínez-Gómez, & Hudson, 2008; Bautista, García-Torres, Prager, Hudson, & Rödel, 2010; Bautista, Zepeda, et al., 2015; Rödel, Bautista, et al., 2008).

The above studies suggest that the comparatively lower body-surface-to volume ratio of pups with relatively lower body mass together with their relatively lower reserves of brown adipose tissue are major drivers of their greater display of behaviors directed to reaching the center of the litter huddle in comparison to their heavier siblings (Bautista, Castelán, Pérez-Roldán, Martínez-Gómez, & Hudson, 2013; García-Torres, Hudson, Castelán, Martínez-Gómez, & Bautista, 2015). Furthermore, a finer-scaled study using infrared thermography has shown that relatively lighter rabbit pups experienced greater benefit in terms of increased peripheral body temperature when they actively increased their number of neighbors by rooting and climbing toward the center of the huddle (Bautista et al., 2017). Overall, these findings underline that relatively lighter and heavier pups generally differ in their thermogenic capacity during early postnatal life, thus leading to differences among siblings in competition for thermal resources within litter huddles. However, detailed information on such individual differences in behavior among littermates, and in particular in species other than the European rabbit, are still scanty (but see: Harshaw & Alberts, 2012; Kammersgaard, Pedersen, & Jørgensen, 2011).

Thus, it was our aim in the present study to explore this purported link between sibling differences in early body mass, individual differences in thermogenic performance during early life, and competitive interactions among siblings in the litter huddle. The study was carried out in house mice *M. musculus domesticus* of wild origin. Although the mouse is one of the most widely used mammalian species in laboratory-based research, studies are usually conducted on inbred strains or otherwise genetically modified animals, with little information available on the "baseline", naturally evolved ancestor (see discussions in: Kavelaars, Heijnen, Tennekes, Bruggink, & Koolhaas, 1999; Koolhaas, 2008; Rangassamy et al., 2016). In a first step we therefore explored whether individual variation in body mass within litters of this species is large enough to detect hypothesized accompanying differences in thermogenic performance among litter siblings, and how such differences develop during early postnatal life. For this, pups were separately exposed to a thermal challenge on different postnatal days and the fine-scale dynamics of the change in their peripheral body temperature was recorded using infrared thermography. In a second experiment, based on the same individuals, we tested for the association between early body mass and the pups' behavior in the huddle. We predicted that relatively lighter pups with a lower thermogenic performance would show a greater effort in terms of rooting and climbing directed toward their littermates, presumably to obtain a thermally more advantageous position within the huddle (cf. Bautista et al., 2017 for such a dynamic in the domestic rabbit).

2 | MATERIALS AND METHODS

2.1 | Study animals

House mice from our breeding stock were fifth generation descendants of animals caught from the wild at different locations near Lyon, France (see details in Forestier, Féron, & Gouat, 2018). We used eight male-female pairs producing a total of 12 litters included in the study (maximum: two litters per pair). Animals were kept on a 14 hr light (white light)/10 hr dark (red light) cycle (white lights off at 12:00 hr) in

standard polycarbonate cages (16.3 × 36.5 × 20.7 cm, Iffa Credo, Lyon, France), containing wood shavings as bedding, and with free access to standard rodent diet (Special Diet Services type M20, Witham, Essex, UK) and water. Temperature was maintained at 21 ± 0.5°C, and relative humidity at approximately 50%. Cages were checked daily for the presence of litters, and when a litter was found this was considered as postnatal day 1. Animals were provided with a large cotton wool ball (approximately 5 cm diameter), which they used for nest building in a corner of their cage. Parental pairs were transferred to clean cages several days prior to parturition.

2.2 | Experiments and procedures

On postnatal day 2, litters were culled to six randomly chosen pups from the 12 litters (72 pups in total, 35 females, 37 males). Sex of individuals was confirmed by genital inspection at weaning on postnatal day 23. Pups were weighed to the nearest 0.01 g and marked with different symbols on their back using a waterproof, nontoxic black felt pen to distinguish them individually from their littermates. Experiments and observations were carried out between 13:00 and 17:00 hr during the animals' active period (Hawkins & Golledge, 2018), that is, during the dark (red light) phase. Individuals were weighed (see below) and returned to their home cages immediately after each test.

2.2.1 | Body mass

Body mass was measured on postnatal days 2–10. Animals were weighed to the nearest 0.01 g on a digital balance and individual symbols were remarked. Given that we were interested in individual differences in body mass within litters, we took within-litter variation into account by calculating the deviation from the litter mean of each pup (in grams). We began testing pups on postnatal day 3 so as not to disturb very young animals and their parents.

2.2.2 | Thermal challenge

As a result of their small size and lack of insulation, new born mice quickly become cold when kept individually at temperatures below 30°C (Lagerspetz, 1962). Considering this and to minimize the disturbance of the parents, individual thermic responses of the animals were measured for 5 min (see details below) on postnatal days 4, 6, 8, and 10. Siblings were separated from their parents and littermates and were immediately put individually in small plastic boxes lined with flannel; on postnatal days 4 and 6, size 4 × 4 × 5.5 (height) cm, on postnatal days 8 and 10, size 8 × 4 × 5.5 (height) cm. As for the recording of huddling behavior (see below), a heating pad (MHP-E1220, ANPAN, China) set to 35°C was placed under the boxes 15 min before the experiments to allow the temperature of the boxes to reach between 22 and 23°C.

2.2.3 | Peripheral body temperature

All pups of the same litter were filmed for 5 min per session with an infrared thermal camera (T650sc, Flir Systems, Boston, MA, USA;

resolution: 640 × 480 pixels, sensitivity of 0.03°C, with emissivity fixed at 0.95, 30 frames per s; camera mounted 55 cm above the apparatus). The 5-min digital recordings were converted into csv format using the software Flir ResearchIR in order to import them into the program R, version 3.4.1 (R Core Team, 2017). Each video frame corresponded to a 640 × 480 matrix of thermal values, which was displayed in R as an image with a gradient of colors corresponding to the temperature of each matrix cell. A single video frame was then captured for analysis each 20 ± 5 s, selected when the pup was well positioned, optimally lying on its ventrum with the back exposed, and not on its side (Harshaw & Alberts, 2012). This resulted in a total of 15 frames chosen for analysis for each 5-min recording. A rectangle was set enclosing each pup so as to capture thermal values corresponding to the pup and its immediate background. The selection of the thermal values excluded all background values that exceeded those of the pup itself. This information was saved to file for each frame and pup. We then chose the highest thermal value per pup, which was usually located in the interscapular region, and this value was taken as the maximum peripheral body temperature of the pup and was used for later statistical analysis. Highest peripheral (body surface) body temperature has been previously found to be a good proxy for individual differences in body temperatures associated with pups' microenvironments within the litter huddle (Bautista et al., 2017; Harshaw & Alberts, 2012). Of the 4,320 possible measurements of highest peripheral body temperature (fifteen 20-s intervals for 72 pups measured on 4 postnatal days), we obtained 3,559 satisfactory measurements for further statistical analysis. The remaining 761 measurements were either discarded due to inappropriate posture of the pups (e.g., lying on their side, on their back or against a wall of the box) or due to technical problems during recording.

To assess the pups' thermogenic performance when separated, we calculated the individual linear regression slopes describing the changes in their peripheral body temperatures during the first 4 min of isolation. This was done because during this period the changes in temperature showed a largely linear shape, at least at the population level (see Figure 1). Furthermore, we used the averaged values measured during the last (5th) min, which we expected to most strongly reflect individual differences in heat loss.

2.2.4 | Interactions in the litter huddle

Huddle behavior of litter siblings was recorded for 10 min during the dark phase (red light) at 13:00 hr on postnatal days 3, 5, and 7. Animals were separated from their parents and placed in a flannel-lined circular acrylic arena (diameter 12 cm) without nest material to allow behavioral recording. A video camera (Sony HDR-XR 200) in night vision mode was mounted 55 cm above the arena to record the pups' behavior. Fifteen min before the experiment, a heating pad (MHP-E1220, ANPAN, China) set to 35°C was placed beneath the arena. This allowed a constant temperature to be maintained in the arena between 22 and 23°C, registered with an electronic thermometer (Digital Thermometer GTH 175/MO; Greisinger Electronic, Germany) located 1 cm above the floor of the arena. This
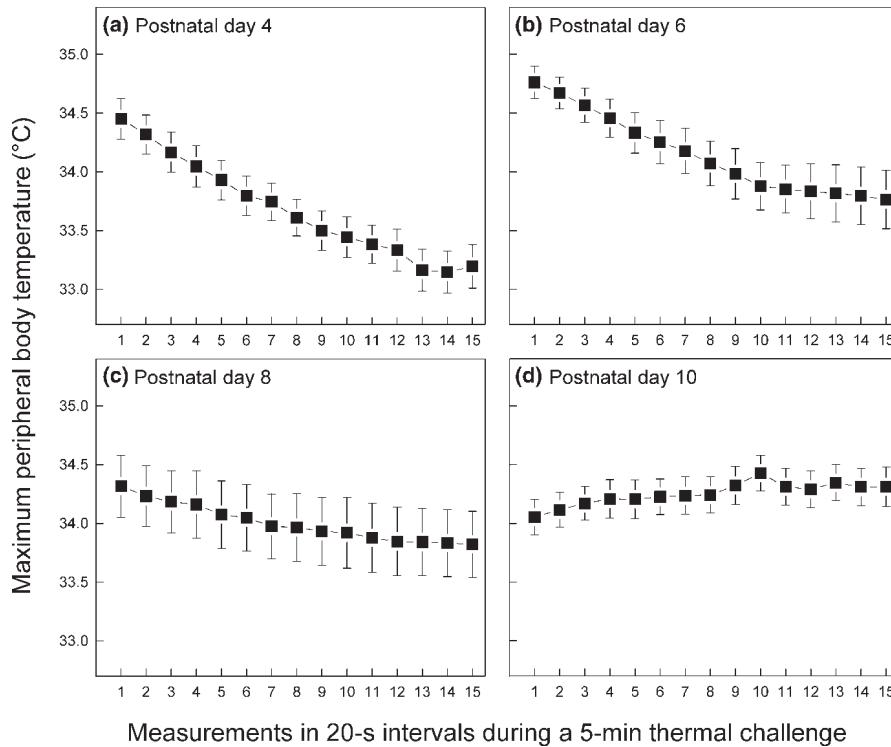
FIGURE 1 Changes in maximum peripheral body temperature in response to a thermal challenge. Means and 95% confidence intervals are given. Pups from 12 litters (n = 6 pups per litter, $n$total = 72) were put separately into small boxes at room temperature (21 ± 0.5°C) for 5 min on different postnatal days and the dynamic of their peripheral body temperature was recorded using an infrared thermal camera. See text for statistics

temperature is considerably below the thermal neutral range of 2-day-old mouse pups, inducing them to huddle, although not so low as to threaten their survival (Gordon, 1993; Lagerspetz, 1962).

We used the software BORIS (Behavioral Observation Research Interactive Software; Friard & Gamba, 2016) to score the following behaviors for each pup in each litter: (a) the duration (in seconds) a pup was huddling, that is, when it was in body contact with at least one littermate with at least 10% of its body surface (cf. Bautista et al., 2010; Reyes-Meza et al., 2011 for similar methods applied to laboratory rats and to domestic rabbits); (b) the duration (in seconds) of rooting, defined as a pup pushing its head between or beneath other pups while vigorously scrabbling with its forelegs and thrusting with its hind legs, and (c) the duration (in seconds) of climbing, defined as a pup placing its forequarters over another pup(s) while thrusting with its hind legs whether or not this resulted in displacement across the top of the huddle (cf. Bautista et al., 2008; Rödel, Bautista, et al., 2008). For analysis, we calculated the percentage of the total 10 min observation time each pup was in body contact with at least one littermate. We summed the durations of rooting and climbing and calculated the percentage time of these behaviors as a function of the total time a pup spent huddling.

2.3 | Ethics statement

Animals were kept and treated according to the ethics and animal care guidelines of France (where the experiments were carried out), and experimental procedures were approved by the local authority for laboratory animal care and use (Comité d'Ethique en Expérimentation Animale Charles Darwin; #7,585 201,610,121,409,165 v2). After weaning on postnatal day 26,

animals were killed in accordance with French animal law. For this, they were first anaesthetized by putting them into a closed transparent plastic box filled with isoflurane gas at a concentration of 3% (IsoFlo, Axience, France), administered by an automatic system (Univentor 400 Anaesthesia Unit, Univentor Ltd, Zejtun, Malta). The anaesthetized individuals were then killed by placing them in a closed transparent plastic box filled with a high concentration of CO_2 gas (delivered by a compressed gas cylinder) for at least 5 min. They were observed until all muscle activity and other signs of life were absent for at least 30 s. All procedures were conducted by a qualified and experienced person. Breeding pairs were kept in the animal facilities for future reproduction.

2.4 | Statistical analysis

Statistical analyses were done using the program R, version 3.4.1 (R Core Team, 2017). We applied linear mixed-effects models (LMM) based on restricted maximum likelihood estimates using the package *lme4* (Bates, Maechler, Bolker, & Walker, 2015). These were used to test for changes across time in peripheral body temperature in response to thermal challenge, and for correlations of sibling differences in body mass with individual slopes describing the body-temperature dynamics in response to a thermal challenge and with the pups' interactions within the huddle. Sibling differences in body mass were calculated as the deviation from the litter mean (Bautista, Zepeda, et al., 2015; Rödel, Bautista, Roder, Gilbert, & Hudson, 2017). We always included litter identity (n = 12 litters) and parental identity (n = 8 pairs) as random factors (random intercepts) in the analysis to take into account these two sources of same origin of

siblings, and thus to avoid an increased risk of false positive results (Williams, Carlsson, & Bürkner, 2017). We also included sex as a fixed factor, in particular as some studies in rodents indicate a higher thermogenic capacity in female pups (Harshaw et al., 2014; Valle, García-Palmer, Oliver, & Roca, 2007).

We checked all LMMs for homogeneity of variances and goodness of fit by plotting residuals versus fitted values. Normality of the model residuals was assured by checking normal probability plots (Faraway, 2005). *P* values for LMM were calculated by *F*-tests based on Satterthwaite's approximation (Bolker et al., 2009). Analysis was based on normalized (scaled) data. Regression slopes (β) with standard errors are provided as a measure of standardized effect size.

In addition, we tested for repeatabilities of certain variables measured across different days during early postnatal life, calculated as the proportion of phenotypic variation that can be attributed to between-subject variation (Lessells & Boag, 1987). These variables, as described above, were: within-litter sibling differences in scaled body mass from postnatal days 3–10 (measured in daily intervals), the scaled individual slopes in response to the thermal challenge tests across postnatal days 4–10, and individual differences within litters in the percentage time the animals showed rooting and climbing behavior in the huddle. Scaled values (body mass, intercepts) were used to account for the increase in absolute variation (in grams) across age (see Figure 2). Calculations were done by LMM-based repeatability using R package *rptR* (Stoffel, Nakagawa, & Schielzeth,

2017), using individual identity as a random factor. Litter identity and parental identity were included as further random factors, and sex was included as a fixed factor. *P* values were calculated by 1,000 Monte-Carlo permutations.

All tests were 2-sided and we used alpha = 0.05 as the level of significance. *P*-values smaller than 0.10 are referred to as "statistical tendencies."

The analysis of the video recordings of huddling behavior and the analysis of thermal recordings were conducted independently in different laboratories (in Mexico and in France) by different people who were naïve at the time of analysis as to the identity of individual pups.

3 | RESULTS

3.1 | Consistency of within-litter differences in body mass

The average body mass of pups was 2.18 g (CI $_{95\%}$: 2.12, 2.25 g) on postnatal day 3 and 5.54 g (CI $_{95\%}$: 5.40, 5.68 g) on postnatal day 10, at the time when the pups usually open their eyes. There was highly significant consistency of body mass differences within litters from postnatal day 3–10 (daily intervals; LMM-based intraclass correlation with 1,000 permutations: $R = 0.764$, $p < 0.001$). More details on within-litter variation in body mass can be found in Table S1 in Supplementary Material.
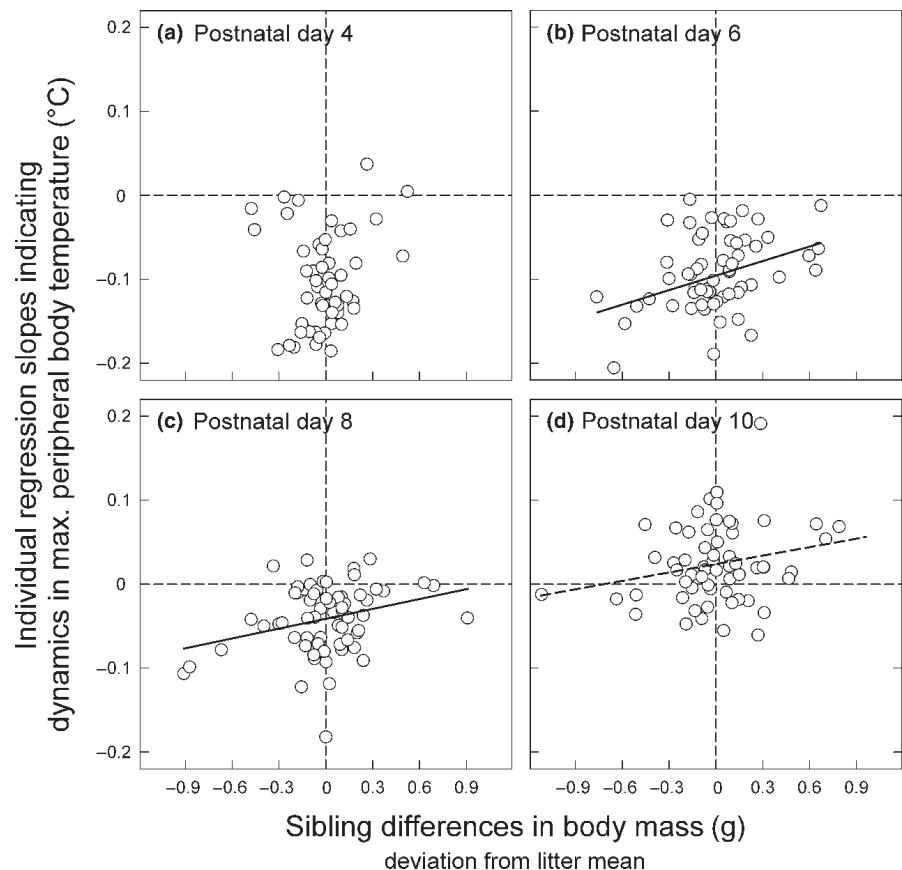

FIGURE 2 Correlations between sibling differences in body mass and the individual regression slopes describing the decrease (see Figure 1a, b, c) or increase (see Figure 1d) in pups' peripheral body temperature in response to a thermal challenge on different postnatal days (72 pups from 12 litters). Body masses were always measured shortly after testing on that postnatal day; sibling differences in body mass were calculated as the deviation from their litter mean. The correlations in (b, c) are statistically significant (solid regression lines), and the correlation in (d) is a statistical tendency (*p* < 0.1; dotted regression line). See text for details of statistics.

3.2 | Dynamics of body temperature in response to thermal challenge

3.2.1 | Changes during early postnatal life

The maximum peripheral body temperatures of the pups decreased significantly during the 5-min thermal challenge (from interval 1 to 15; Figure 1) by on average –1.210°C (CI $_{95\%}$: –1.401, –1.018°C) on postnatal day 4 (LMM: $F_{1,89}$ = 131.280, p <0.001), by on average –1.107°C (CI $_{95\%}$: –1.166, –0.869°C) on postnatal day 6 ($F_{1,104}$ = 114.700, p < 0.001), and by on average –0.496°C (CI $_{95\%}$: –0.615, –0.377°C) on postnatal day 8 ($F_{1,115}$ = 31.292, p < 0.001). However, there was a significant increase in maximum peripheral body temperatures on postnatal day 10 by on average 0.258°C (CI $_{95\%}$: 0.098, 0.418°C; $F_{1,104}$ = 7.882, p = 0.006).

The changes in peripheral body temperatures across time did not differ significantly between males and females for any of the postnatal days tested (all p > 0.10). However, females had significantly higher peripheral body temperatures than males on postnatal day 4, by on average 0.162°C ($F_{1,92}$ = 4.503, p = 0.037). There were no such significant sex-specific differences on postnatal days 6, 8 or 10 (all p > 0.10).

We tested for the repeatability of the individual slopes in response to the thermal challenge tests across postnatal days 4–10. Results revealed that such individual differences were significantly consistent across time (LMM-based intra-class correlation with 1,000 permutations: R = 0.129, p = 0.005). More details on the variation among these individual regression slopes can be found in Table S2 of the Supplementary Material.

3.2.2 | Differences between heavier and lighter pups

We first calculated the slopes of individual pups' temperature dynamics during the first 4 min of the thermal challenge (20-s intervals 1 to 12; see below for results of the last, 5th, test min), expressing the degree of decrease (mostly during postnatal days 4, 6, 8; Figure 2a–c) or increase (mostly during postnatal day 10; Figure 2d) in their maximum peripheral body temperature. These slopes were significantly and positively associated with sibling differences in the pups' individual body mass on postnatal day 6 (LMM: $F_{1,49}$ = 22.739, β = 0.348 ± 0.073 SE, p < 0.001; Figure 2b), postnatal day 8 ($F_{1,54}$ = 7.014, β = 0.309 ± 0.167 SE, p = 0.010; Figure 2c), and barely missing the level of statistical significance on postnatal day 10 ($F_{1,49}$ = 3.719, β = 0.241 ± 0.125 SE, p = 0.059; Figure 2d). This indicates that relatively lighter pups experienced a steeper decrease in their body temperature during the course of the first 4 min of the thermal challenge on postnatal days 6 and 8. On postnatal day 10 relatively lighter pups tended to show a less steep increase in body temperature during the thermal challenge. However, on postnatal day 4 there was no significant association between pups' individual slope and their relative body mass ($F_{1,43}$ = 2.198, β = 0.112 ± 0.076 SE, p = 0.146; Figure 2a).

In addition, the maximum peripheral body temperatures of the animals during the last minute (min 5; averages of 20-s intervals 13–15; cf. Figure 1) were significantly and positively associated with individual body mass relative to siblings on postnatal day 6 (LMM: $F_{1,44}$ = 6.646, β = 0.177 ± 0.069 SE, p = 0.013), and with a statistical tendency (p < 0.10) on postnatal day 4 ($F_{1,39}$ = 3.776, β = 0.225 ± 0.116 SE, p = 0.059) and postnatal day 10 ($F_{1,54}$ = 3.420, β = 0.219 ± 0.119 SE, p = 0.070). There was no indication of such a trend on postnatal day 8 ($F_{1,49}$ = 0.514, β = 0.078 ± 0.109 SE, p = 0.476; see Figure S1 in Supplementary Material).

The relative maximum body temperatures measured at the onset of the thermal-challenge experiment (measured during 20-s interval 1; cf. Figure 1) were not significantly associated with the relative body masses of pups on postnatal days 4, 6, 8 and 10 (all p > 0.05). Furthermore, there were no significant differences between male and female pups for any of the postnatal days (all p > 0.10).

3.3 | Interactions in the litter huddle

During the 10 min after the litter was placed into the test arena on postnatal days 3, 5, and 7, pups were in contact with at least one sibling for on average 67.0% of the time (CI $_{95\%}$: 61.9%, 72.0%). For on average 3.2% of this time (CI $_{95\%}$: 2.7%, 3.7%) pups repeatedly rooted under and climbed over their adjacent sibling(s) (more details in Table S3 in Supplementary Material).

The percentage time individual animals showed rooting and climbing—relative to their litter siblings—was repeatable at the individual level across postnatal days 3, 5, and 7 (LMM-based intraclass correlation with 1,000 permutations: R = 0.290, p < 0.001).

3.3.1 | Differences between heavier and lighter pups

The relative body mass of pups (with respect to their litter mean) on postnatal day 3 (i.e., at the first time of measurement and of individual marking) was significantly and positively associated with the percentage time they spent in body contact with siblings (LMM: $F_{1,59}$ = 4.169, β = 0.140 ± 0.069 SE, p = 0.045; Figure 3a), and was significantly and negatively associated with the percentage time they showed rooting and climbing ($F_{1,59}$ = 6.105, β = −0.230 ± 0.093 SE, p = 0.016; Figure 3b). That is, relatively lighter pups spent a lower percentage time in huddling groups than their heavier siblings. Once they were in a huddle they spent a higher percentage of that time rooting under and climbing over their littermates. There was no significant difference between male and female siblings with respect to either of the behavioral variables tested (both p > 0.10).

3.3.2 | Thermogenic performance and behavior in the litter huddle

The percentage time pups showed rooting and climbing towards their siblings was significantly and negatively associated with the
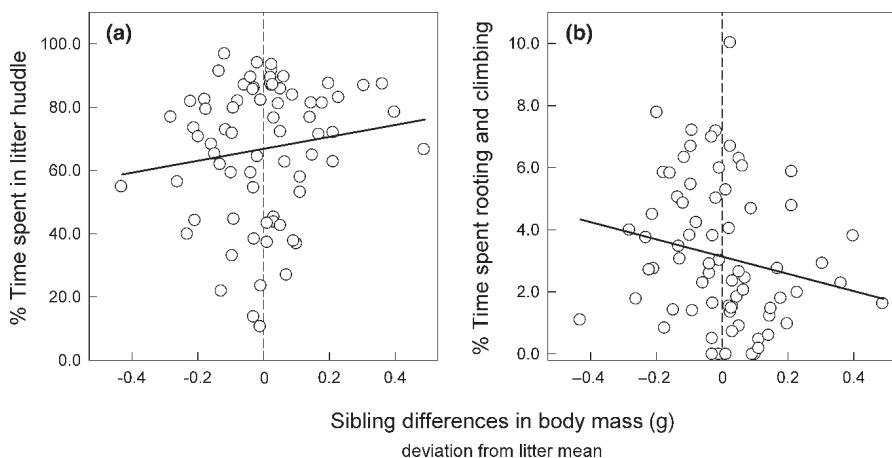
FIGURE 3 Correlations between relative differences among siblings in body mass (measured as the deviation from litter means on postnatal day 3) and (a) the percentage time the animals spent in body contact with at least one litter sibling and (b) the percentage of contact time they spent rooting under and climbing over their siblings. Data points are averages of repeated measurements of 72 pups from 12 litters on postnatal days 3, 5, and 7. Behaviors were recorded after the whole litter was transferred to a test arena for 10 min. Both correlations are statistically significant; regression lines are based on parameter estimates of LMM given in the text

individual slopes describing the dynamics of their decrease in peripheral body temperature during the thermal challenge on postnatal days 4, 6 and 8 (LMM: $F_{1,69}$ = 7.350, β = −0.296 ± 0.109 SE, p = 0.008). That is, pups with an apparently lower thermogenic performance—as indicated by a steeper decline (= lower negative slope values; cf. Figure 2) in their peripheral temperature—showed significantly more directed behaviors towards their siblings than pups with a higher thermogenic performance.

4 | DISCUSSION

Based on the present results we can report that house mouse pups show stable within-litter differences in body mass during the early postnatal period, and that these differences are positively correlated with differences in thermogenic performance in response to cold challenge. As predicted, relatively heavier pups maintained peripheral body temperature more strongly than their lighter litter siblings when confronted with such a challenge. Furthermore, relatively lighter siblings with an apparently lower thermogenic performance showed a greater effort in terms of rooting and climbing directed towards their littermates.

During the early postnatal period altricial young have an incomplete ability to thermoregulate physiologically, and energy resources are predominantly allocated for early growth and development (Hull, 1973). Studies of the ontogeny of homeothermy in various species of altricial rodents indicate that the young are able to thermoregulate independently at around the time when they have developed a sufficient fur cover, usually around the time of eye-opening (Couture, 1980; Gebczynski, 1975; Hill, 1976, 1983 ; McManus, 1971; Soholt, 1976)—which is at around postnatal days 11–13 in the house mouse (Williams & Scott, 1953). This general assumption is well supported by our detailed

measurements of the peripheral body temperature dynamics of wild-derived house mouse pups at an ambient temperature of 22–23°C, showing stable temperature profiles on postnatal day 10 when isolated from parents and littermates. Under such conditions, mouse pups of younger age (postnatal days 4, 6, 8) were not able to maintain a stable body temperature (Harshaw et al., 2014), and as could be expected, decreases in body temperature were more pronounced the younger the pups were (see studies on different altricial rodents: Couture, 1980; Cutrera, Antinuchi, & Busch, 2003; Gebczynski, 1975; Harshaw & Alberts, 2012). Nevertheless, our findings show that within a certain age class, relatively heavier pups showed less pronounced temperature losses (postnatal days 6, 8) or even tended to stabilize their peripheral body temperatures more efficiently (postnatal day 10) compared to their lighter littermates. However, on postnatal day 4 there were no indications for such an association (Figure 2a), possibly because all pups still had sufficient reserves of brown adipose tissue to similarly defend their body temperature against the cold challenge (cf. García-Torres et al., 2015). These differences between heavier and lighter pups on postnatal days 6 to 10 could be due mainly to the more favorable body surface-to-volume ratio in relatively heavier pups. Furthermore, as has been shown in studies of domestic rabbits, this might be also due to the generally quicker depletion of brown adipose tissue and thus smaller remaining reserves of such in relatively lighter pups, restricting those individuals in their ability to efficiently rely on nonshivering thermogenesis (Bautista et al., 2013; García-Torres et al., 2015). Our findings also support a slightly higher thermogenic capacity in female mouse pups at least during the very early postnatal period, broadly confirming the results of another recent study on sex-specific differences in thermogenesis of young laboratory mice (Harshaw et al., 2014). This sex difference might be possibly due to a generally greater brown adipose tissue

thermogenesis in females as has been reported in studies of laboratory rats (Rodriguez-Cuenca et al., 2002; Valle et al., 2007).

Knowledge of the dynamics of body temperatures in response to thermal challenge during the early postnatal days is particularly important as during this period the young are highly vulnerable to such environmental fluctuations and mortality, including from hypothermia, is often high (Millar, 2007; Rödel et al., 2009). For example, the lower capacity to maintain body temperature in lighter young is discussed as an important mechanism explaining the link between low birth mass and low growth and survival in domestic piglets *Sus scrofa domesticus* (Cutler, Fahy, Cronin, & Spicer, 2006). Furthermore, our findings underpin the existence of consistent individual differences in the thermogenic capacity of altricial young relative to littermates across early postnatal life (see Rödel et al., 2017 for European rabbits). It remains to be investigated whether such consistent sibling differences during early development will exert priming effects on the animals' metabolic responses to cold challenges during later life (cf. Morrison, Ramamurthy, & Young, 2000; Vollmer & Skøtt, 2002).

In the second part of our study we explored the association between the pups' relative differences in thermogenic performance as assessed by their changes in peripheral body temperature during isolation, and their propensity to actively try to gain a thermally favorable position within the litter huddle. The tendency of pups to huddle together with siblings during early life is a prominent feature in polytocous altricial mammals in order to maintain a high and stable body temperature by reducing heat loss and thus lowering an individual's energy expenditure for thermoregulation (Gilbert et al., 2012, 2010 ; Hudson et al., 2011; Hull, 1973)—and such effects have been described in laboratory mouse pups as well (Harshaw & Alberts, 2012; Stanier, 1975). However, huddling together with siblings is a dynamic process characterized by frequent changes in the pups' positions relative to littermates, reflecting their competition for thermally advantageous positions in the center of the huddle (Alberts, 1978; Bautista et al., 2008). The findings of our study are also consistent with our prediction that in particular the relatively lighter pups with a relatively lower thermogenic capacity would work harder, that is, show more rooting and climbing behavior in the apparent attempt to increase insulating contact with littermates, as has been shown in previous studies in domestic rabbits and laboratory rats (review in Hudson et al., 2011). Furthermore, relatively lighter pups generally spent less time in contact with their siblings, presumably due to their higher probability of being forced to occupy peripheral positions and even to temporarily loose contact with the huddle (cf. Bautista, Zepeda, et al., 2015). Overall, the findings and their high similarity to the patterns observed in the better studied domestic rabbit (Hudson et al., 2011) suggests the existence of broad commonalities in individual differences among littermates with respect to the behavioral and physiological processes accompanying early development in polytocous altricial mammals.

5 | CONCLUSIONS

The simple, noninvasive method used here (infrared thermographic imaging) to record peripheral body temperature was sufficiently sensitive to detect fine changes in the development of the response to thermal challenge across early development in the house mouse (see also Harshaw & Alberts, 2012). These changes, measured in individual pups separated from their littermates, were also sufficient to determine consistent individual differences in their thermogenic capacity. In turn, these individual differences were predictive of an individual's interactions with litter siblings, as pups with a relatively lower thermogenic capacity showed a consistently higher propensity to initiate directed behaviors towards their siblings apparently to achieve a thermally more favorable position within the litter huddle. Given what seems to be such a stable and conserved set of behavioral and physiological responses to cold exposure, a presumably common and elemental environmental threat, we can ask what might be the consequences of this for later life, both at physiological and behavioral ("personality") levels. First indications that such early differences in directed behaviors to reach a central position within a litter will translate into differences in behavioral types during later life come from a study on domestic rabbits (Reyes-Meza et al., 2011). Long-term studies exploring how the different micro-niches individuals occupy during early development might contribute to shaping stable physiological and behavioral differences in later life—and such effects might even emerge in inbred or genetically identical strains of laboratory animals (Freund et al., 2013; Lewejohann, Zipser, & Sachser, 2011; Prager, Stefanski, Hudson, & Rödel, 2010)—are particularly worthwhile given the importance of the mouse in biomedical and psychobiological research.

CONFLICTS OF INTERESTS

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

IX. Discusión general

Nuestros resultados mostraron las relaciones entre los mecanismos conductuales y fisiológicos presentes en la conducta termorregulatoria durante la primera semana de vida, y sus efectos sobre las diferencias individuales en conducta y fisiología a corto y mediano plazo en dos especies de mamíferos altriciales. Para el conejo y parcialmente para el ratón doméstico, algunos de los mecanismos que están presentes durante la conducta termorregulatoria dentro del nido son el peso al nacimiento, las conductas dirigidas "rooting y climbing", el número de hermanos en contacto, la cantidad de leche ingerida, la conversión de leche a biomasa y la temperatura corporal del individuo. Aquellos individuos más pesados al nacimiento tienen un mayor número de hermanos en contacto durante el agrupamiento, presentan una mayor temperatura corporal, ingieren más leche, ganan más peso y sobreviven más. Los mecanismos presentes durante el ambiente desarrollo temprano en el conejo doméstico que describimos y sus relaciones coinciden con los trabajos publicados anteriormente por nuestro grupo de investigación (Rödel y cols. 2008a; Hudson y cols. 2011; Bautista y cols. 2015a).

El ambiente de desarrollo temprano es un factor que predice las trayectorias de vida de los individuos en diferentes especies de animales (Lindstöm 1999). Esto coincide con nuestro ya que en ambas especies la termorregulación conductual durante las primeras semanas de vida es crucial para su crecimiento y supervivencia (Bautista y cols. 2003; Harshaw y Alberts 2012), aquellos individuos que se agrupan destinan más energía a su crecimiento y posterior supervivencia (Bautista y cols. 2003; Gilbert y cols. 2007). Confirmamos que estas diferencias en las trayectorias de vida de los individuos son moduladas a través de una serie de mecanismos fisiológicos y conductuales durante su desarrollo temprano. En congruencia con previos estudios en el conejo doméstico (Bautista y cols. 2003, 2005, 2008, 2013, 2015a, 2015b; García-Torres y cols. 2015; Reyes-Meza y cols. 2011) nuestros hallazgos confirman que para estas crías de mamíferos altriciales el peso al nacimiento dirige dicha secuencia de mecanismos (Fig. 1 Panel a y b en el capítulo 2). Asimismo, estos mecanismos mostraron generalidad y convergencia, ya que a pesar de que utilizamos dos modelos animales con distintos ambientes de desarrollo temprano, en el roedor la variación intra-camada inicial en el peso corporal moduló

tanto la respuesta termogénica ante un reto térmico, como los esfuerzos por acceder al centro del agrupamiento, como se había reportado anteriormente en ratas (Bautista y cols. 2009) y conejos (Bautista y cols. 2008), cabe resaltar que para el ratón doméstico, este es un hallazgo que por primera vez enmarca a la ontogenia de la homotermia como un producto de las diferencias individuales entre hermanos.

En nuestro trabajo con el ratón no evaluamos el efecto materno sobre el desarrollo de las diferencias individuales en conducta y fisiología. Pero, considerando que en roedores la interacción que tienen las crías con la madre desde la etapa intrauterina es un factor capaz de modular su conducta y fisiología a corto plazo y largo plazo (Waterland y Jirtle, 2003), nuestros hallazgos coinciden con lo reportado en roedores y conejos sobre el efecto que tiene el ambiente intrauterino (madre-crías) sobre el peso de los individuos (Mendl 1988; Bautista y cols. 2015b), en donde la restricción de espacio y nutrientes tienen efectos negativos a corto plazo sobre el peso al nacimiento y el crecimiento (Wehmer y Jen, 1974). En este trabajo tampoco evaluamos si estas diferencias individuales intra-camada en peso prevalecen en edades posteriores, pero, otros investigadores mostraron que el peso corporal en el día 2 y la ganancia de peso al día 20 se relacionan negativamente con el tamaño de camada (Mendl 1988; Hermann y cols. 2009) en roedores, por lo que podríamos suponer que estás diferencias individuales son consistentes hasta a alcanzar el destete.

Esto concuerda con lo reportado en conejo, donde los individuos más pesados al nacimiento provienen de camadas pequeñas, crecen más y tienen más probabilidades de sobrevivir hasta edades posteriores de su vida en comparación con aquellos ligeros, sumado a lo anterior, los individuos que sobreviven durante las primeras semanas de vida se benefician de la muerte de sus hermanos ya que obtienen una mayor cantidad de leche durante el amamantamiento (Drummond y cols. 2000). Si bien esto ya ha sido descrito en trabajos previos, nuestro hallazgo mediante un análisis multivariado (*confirmatory path analysis*) nos permitió verificar el ajuste de hipótesis causales, así como identificar la contribución directa e indirecta de un conjunto de variables a la variación de una respuesta (Shipley, 2009) por primera vez reportamos que junto con el tamaño de camada al nacimiento, la reducción de la camada por

muerte de sus miembros y el peso al nacimiento, la interacción intra-camada durante la conducta termorreguladora (número de vecinos en contacto) en los primeros día de vida explica parte de la variabilidad en el peso al destete en las camadas del conejo doméstico como un efecto indirecto del peso al nacimiento.

Nuestros hallazgos en conjunto explican el origen de las diferencias individuales en conducta y fisiología y sus efectos a corto y mediano plazo para los individuos en dos especies de mamíferos altriciales evaluadas en condiciones controladas de laboratorio. Sin embargo, en la naturaleza, estás especies se enfrentan a condiciones variantes y en su mayoría adversas. Por ejemplo, en condiciones semi-naturales los nidos del conejo europeo se encuentran expuestos a temperaturas del suelo inferiores a los 21 °C. (Rödel y cols. 2008b), esta condición térmica expone a las crías a una temperatura ambiental por debajo de su ZTN (Hull, 1965). Esta condición natural junto con el peso al nacimiento y el tamaño de camada son limitantes para el desarrollo y supervivencia de los individuos durante los primeros siete días de vida (Rödel y cols. 2009a). Con base en esto, nuestros hallazgos aportan una descripción más detallada de los mecanismos que modulan las diferencias individuales en desarrollo y supervivencia en esta edad crítica. Este supuesto se ve reforzado porque durante la primera semana de vida la conducta maternal y de las crías es similar tanto en condiciones de laboratorio como en condiciones naturales (Zarrow y cols. 1965; Hudson y Distel, 1982; Jilge y Hudson, 2001). Es decir, a pesar de la domesticación, nuestros hallazgos en estos modelos tienen significado biológico para la historia natural de la especie.

En este sentido, algo que no consideramos y podríamos medir en el futuro es el efecto de la estacionalidad, en la que nacen los individuos, sobre la ontogenia de las diferencias individuales y el efecto que puede tener sobre su éxito reproductivo. Existen reportes en condiciones semi-naturales indicando que aquellos individuo nacidos al inicio de la temporada reproductiva tienen una mejor condicion corporal (mayor peso corporal) y como consecuencia sobreviven más (Rödel y cols. 2000). En adición, Los individuos más pesados (que provienen de camadas pequeñas) en la población normalmente sobreviven más y, en el caso de los machos (con mayor peso corporal en el otoño) son más victorios en los encuentros intra-sexuales

agónisticos asociados posiblemente al establecimiento de la jerarquia social (Rödel y von Holst. 2009). Para las hembras la condición corporal también está en función de la estacionalidad y, al igual que en machos, tienen más posibilidades de sobrevivir si nacen al inicio de la temporada reproductiva (Rödel y cols. 2009b).

Además, junto con la estacionalidad, el tamaño de la camada en que nacieron y la edad de su madre afectan aspectos de su vida reproductiva, específicamente la supervivencia hasta la madurez sexual de su descendencia, ya que esta disminuye mientras más tarde en la temporada reproductiva hayan nacido las madres, aumenta conforme aumenta la edad de la madre y si la madre proviene de una camada de tamaño intermedio de 3 a 5 individuos (Rödel y cols. 2009b). Aunque en el laboratorio las condiciones del bioterio obligan a los individuos a estar en un regimen de luz/oscuridad parecido a la temporada reproductiva, por observaciones personales el tamaño de camada y la supervivencia de esta durante las 2 primeras semanas sigue un patrón similar al reportado en condiciones semi-naturales. Este supuesto, en conjunto con los datos producidos durante este proyecto doctoral pueden dar una aproximación del impacto del ambiente de desarrollo temprano sobre aspectos de la adecuación en hembras del conejo.

Según lo anterior, sabemos que las condiciones ambientales experimentadas durante la vida temprana pueden tener consecuencias a largo plazo sobre la adecuación de los individuos, espeficicamente la interacción temprana entre hermanos descrita por nuestros hallazgos y considerada en los estudios en condiciones semi-naturales. Por ejemplo, en condiciones semi-naturales la supervivencia está en función del tamaño de camada y del ambiente térmico de los nidos. Para esta especie cuando la temperatura ambiental del nido es menor a los 10 °C los individuos ganan más peso si están en camadas medianas (3-4 miembros). Sin embargo, si la temperatura es mayor a los 10 °C la ganancia de peso es mayor si las camadas están compuestas por sólo 2 individuos (Rödel y cols. 2008b) es decir tener e interactuar con más o menos hermanos dentro del nido modula el crecimiento en función de la temperatura ambiental. Uniendo este hecho con lo que sabemos sobre aspectos del éxito reproductivo en hembras del conejo europeo, descritos anteriormente. y lo que ahora sabemos sobre los mecanismos presentes en la termorregulación conductual en la vida posnatal, podemos en futuras investigaciones incorporar el efecto intrauterino descrito ya ampliamente en mamíferos

altriciales (Ryan y Vandenverg, 2002) como un factor que interactúa con los anteriores para dar forma a la variación intra-camada en fisiología y conducta a corto y largo plazo. Esto es posible si consideramos que el peso al nacimiento está definido en parte por el sitio de implantación que ocuparon las crías durante su gestación, las crías más pesadas se situaron normalmente en ambos extremos del cuello uterino (Bautista y cols. 2015b).

En esta tesis evaluamos el efecto de interacción temprana entre hermanos sobre las diferencias individuales en fisiología y conducta a corto y mediano plazo (cerca del destete). Con base en lo anterior, en otra parte de este proyecto doctoral que está en proceso estamos evaluando el efecto de la posición ocupada por las crías dentro del cuello uterino en interacción con el ambiente de desarrollo posnatal temprano y su relación con aspectos conductuales, fisiológicos y reproductivos en hembras adultas del conejo doméstico. En este planteamiento partimos del hecho de que existe un efecto del sexo de los vecinos machos adyacentes sobre la conducta, fisiología y aspectos reproductivos de las hembras. En comparación con hembras que se desarrollaron prenatalmente entre 2 hembras (0M), las hembras que se desarrollaron entre dos machos (2M) presentan una distancia anogenital casi tan grande como la de los machos, y realizan marcas por frotamiento del mentón con mayor frecuencia (Bánszegi y cols. 2009). Esta conducta en particular está asociada al estado reproductivo de las hembras (Hudson y cols. 1990), sin embargo, las hembras 2M son menos atractivas sexualmente (Bánszegi y cols. 2012). Por ello nos preguntamos por el efecto de la interacción entre las condiciones de desarrollo prenatales con los mecanismos posnatales presentes en la conducta termorregulatoria temprana; en primer lugarespecífico sobre el desarrollo de síndromes conductuales o personalidad animal en el conejo doméstico ya que las crías ligeras que en promedio estuvieron en contacto con un menor número de hermanos (periféricas) desarrollan a largo plazo un síndrome conductual proactivo en comparación con sus hermanas pesadas (centrales) tanto en el laboratorio (Reyes-Meza y cols. 2011) como en condiciones semi-naturales (Rödel y cols. 2017).

Los mecanismos físiologicos que subyacen a las diferencias indivuales en conducta que en esta tesis y. en trabajos previos, reportamos son modulados por el peso nacimiento. Este factorsugiere una aproximación por un lado a los factores "genéticos" atribuidos a nacer con un

mayor peso corporal y por otro a los factores ambientales involucrados en la ontogenia de los mamíferos altriciales que crecen en camadas. Por ejemplo, la capacidad termogénica de los individuos depende del peso al nacimiento y de la interacción temprana entre hermanos durante el agrupamiento, las crías más ligeras y que estuvieron en contacto con un menor número de hermanos son menos capaces de mantener una temperatura corporal alta y estable prolongadamente ante un reto térmico agudo o moderado (García-Torres y cols. 2015) esto es debido, en parte, a que nacen con menor cantidad de TAP (Dawkins y Hull, 1964) y a la expresión de la proteína UCP1 diferencial entre las crías inducida por el ambiente térmico experimentado (Bautista y cols. 2013). Sin embargo, aun falta por explorar la relación de la interacción temprana entre hermanos con los efectos sobre hormonas tiroideas, glucocorticoides y catecolaminas. Que por un lado se sabe participan disminuyendo o incrementando la respuesta termogénica del TAP en las crías altriciales (Hull, 1965; Seydoux y cols. 1982; Bianco y Silva, 1987; Xu et al. 2009). Y por otro lado tienen efectos epigenéticos, como en el desarrollo temprano de roedores, donde los glucocorticoides en conjunción con la conducta de la madre, modifican la expresión de receptores para glucocorticoides en el cerebro de las crías con implicaciones sobre la respuesta al estrés en su vida adulta (Weaver y cols. 2004). Así futuras investigaciones pueden evaluar el efecto que tiene esta actividad neuro-endocrina temprana en interacción con los mecanismos presentes en la ontogenia de las diferencias individuales en conducta y fisiología sobre áreas específicas del cerebro. en particular en el área preóptica del hipotálamo, considerada como el centro termorregulador (Nakamura 2011).

En resumen, el ambiente de desarrollo temprano puede predecir las trayectorias de vida a corto y largo plazo en estos mamíferos altriciales (Hudson y cols. 2011). Pero, una aproximación más audaz y enmarcada en teorías como el evo-devo que entre sus objetivos tiene explicar la biodiversidad en términos de trayectorias ontogenéticas (Müller, 2007), nos obliga a preguntarnos por la contribución de la interacción temprana entre hermanos sobre la activación o inactivación de los genes involucrados en el desarrollo las diferencias individuales, además a preguntarnos sobre las señales específicas que durante el desarrollo intra-uterino y posnatal modulan la expresión del fenotipo. Ejemplos de estas aproximaciones las encontramos en

especies de invertebrados como las hormigas, estos trabajos hacen énfasis en la interacción social (mediante hormona juvenil)

como uno de los factores que generan las diferencias físicas al interior del sistema de castas de estos insectos durante el desarrollo temprano (Rajakumar y cols. 2018). Algo similar encontramos en vertebrados, específicamente en mamíferos la restricción de crecimiento intra-uterino -definido como el crecimiento y desarrollo disminuido de los embriones/fetos y órganos de mamíferos durante la gestación, revisado en Mcmillen y Robinson (2005)- ya sea producto de desarrollarse en camadas como el cerdo (Wang y cols. 2008) o conejo (Eixarch y cols. 2012) o experimentadas por la madre en rata (Lillycrop y cols. 2008) y humanos (Hart, 1993) tiene efectos sobre el desarrollo de órganos y desarrollo neuromotor (cerdos y conejos) y es detonante de enfermedades en la edad adulta como diabetes y obesidad en humanos y roedores. Con estos antecedentes podemos, en futuras investigaciones, sugerir que la variación intra-camada mediante la interacción entre hermanos durante la conducta termorregulatoria posnatal juega un papel "epigenético" en la ontogenia de los individuos. Esto puede ser sustentado, por un lado, porque previamente en un estudio mediante *cross-fostering* mostramos que el peso relativo a los miembros de la camada explica diferencias en la ganancia de peso en la primera semana posnatal en gran parte independientemente de las "cualidades genéticas" implícitas que tienen las crías mas pesadas dentro de las camadas (Bautista y cols. 2015a), y por otro lado, porque al nacimiento tanto las crías pesadas como las ligeras no presentan diferencias histologías (número de vacuolas que contienen ácidos grasos) en el TAP (García y cols. 2015) lo que nos hace suponer que el ambiente de desarrollo temprano definido por la interacción entre hermanos es capaz de moldear la variación fenotípica independientemente de factores estocásticos como el sitio de implantación intrauterina o la ventaja "genética" en biomasa que tienen las crías por ser pesadas .

Finalmente, hasta ahora conocemos en parte algunos de los efectos a corto y mediano plazo de los mecanismos involucrados en la interacción temprana entre hermanos durante la termorregulación conductual de crías de mamíferos altriciales sobre el desarrollo de las diferencias individuales en conducta y fisiología. Pero, si nuestros hallazgos son ampliados para investigar a largo plazo (en al edad adulta y la vejez) estos efectos, pueden contribuir a entender

mejor las respuestas adaptativas predictivas, específicamente el fenotipo *ahorrador* (Hales y Barker, 2001) y sus consecuencias sobre los orígenes de la enfermedad en edades adultas.

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ANEXO I

Zepeda, J.A., Rangassamy, M., Monclús, R., Féron, C., Hudson, R., Rödel, H.G., Bautista, A. (2016) Posiciones térmicamente ventajosos dentro del agrupamiento de la camada y la capacidad termorreguladora individual como predictores de conductas relationadas a la ansiedad en *Mus musculus* del tipo salvaje. *Curso International Bases Biológicas de la Conducta*. Tlaxcala, Mexico.

Posiciones Térmicamente Ventajosas Dentro Del Agrupamiento de la Camada Y La Capacidad Termorreguladora Individual Como Predictores De Conductas Relacionadas A La Ansiedad En *Mus musculus* Del Tipo Salvaje

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POSGRADO EN CIENCIAS BIOLÓGICAS

INTRODUCCIÓN

- El ambiente de desarrollo temprano es capaz de moldear las diferencias individuales en conducta y fisiología.
- En crías de mamíferos altriciales se ha reportado que los individuos de una misma camada ocupan diferentes sitios dentro del agrupamiento que forman en el nido. Estas diferencias en la posición dentro del agrupamiento que forman en el nido para termoregular, están asociadas con diferencias en sus capacidades termorregulatorias individuales y con diferencias en sus estilos conductuales en etapas posteriores de su vida.
- Tales resultados se han reportado previamente en ratas y en conejos. Sin embargo, aún no sabemos como las diferencias en las posiciones ocupadas por las crías dentro del agrupamiento y sus capacidades termorregulatorias individuales modifican las conductas relacionadas a la ansiedad en *Mus musculus* del tipo salvaje.

- Objetivo: investigar como el ambiente de desarrollo temprano está asociado a diferencias en conductas relacionadas a la ansiedad en la etapa juvenil en *Mus musculus*

METODOLOGÍA

Posnatal

- Agrupamiento
- Capacidades individuales para termorregular
- Vocalizaciones

Juvenil

- Conductas relacionadas a la ansiedad (EPM)

n= 12 camadas 6 (crías)

Crías de 1 día posnatal (DP)

Crías de 8 días posnatales en la caja nido

Agrupamiento

DP3   DP5   DP7   DP9

Cría Focal	N de contactos	Rooting frecuencia	Climbing frecuencia
1	3	2	4

Capacidades termorregulatorias individuales

DP 4 y 6   DP 8 y 10

vocalizaciones

DP 11 y 12

Laberinto Elevado en Cruz

DP 23 y 26

Medición de CORT DP 26

PREDICCIÓN

Esperamos que existan diferencias en las conductas relacionadas a la ansiedad asociadas a la posición que ocuparon en el agrupamiento, las capacidades termorregulatorias individuales durante los primeros días posnatales.

ANEXO II

Rödel, H.G., Hudson, R., Zepeda, J.A., Reyes, V., Féron, C., Bautista, A. (2017) Body mass modulates huddling dynamics and body temperature profiles in pups of the domestic rabbit. *35th International Ethological Conference IEC*. Estoril, Portugal.

Body mass modulates huddling dynamics and body temperature profiles in rabbit pups

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Introduction

Altricial mammals typically lack the physiological capacity to thermoregulate independently during the early postnatal period, and in litter bearing species the young benefit strongly from huddling together with their litter siblings. Such litter huddles are highly dynamic systems, often characterized by competition for energetically favorable, central positions.

In our study on domestic rabbits *Oryctolagus cuniculus*, we asked whether individual differences in body mass affect changes in body temperature during changes in the position within the huddle. We predicted that pups with relatively lower body mass should be more affected by such changes arising from huddle dynamics in comparison to heavier ones.

Material & Methods

Experiments on domestic rabbit pups were carried out at the Institute of the Centro Tlaxcala de Biología de la Conducta de Universidad Autónoma de Tlaxcala.

Body surface temperature anomalies in 15 litters of potential days 3, 5 and 7. Sibling interactions, e.g. cases of nesting and climbing, in the centre of the litter huddle were studied in 4 boxes in due to rearrange large (non-centered) pups.

Changes in pups' body surface temperatures were measured by comparing 4 repeated measures of infrared thermographs during each set up (Figure 1).

Analyses were done using linear mixed-effects models using litter identity as a random factor. Statistically significant differences at indicated by different letters (Figures 2, 3, 4). Means with 95% confidence intervals are given in the same letters.

Figure 1. Infrared camera view and related thermographic registration of a litter.

Results & Discussion

Figure 2.

(a) lighter pups (b) heavier pups

Changes in max. body surface temperature [°C] vs Changes in numbers of neighbours in the litter huddle
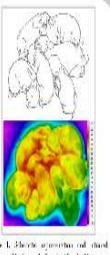
n=135, n=88, n=40, n=157, n=134, n=48

Figure 3.

N neighbours in litter huddle vs Body mass categories of pups within litter

lighter, heavier — n=528, n=448

Figure 4.

Rooting and climbing towards the centre (seconds / 5 min observation time)

lighter, heavier — n=8, n=8
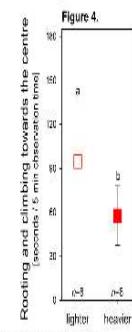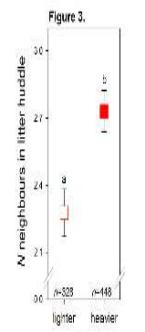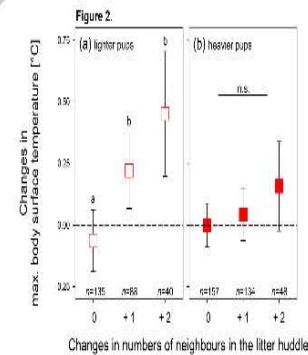
Lighter pups showed significant increases in their maximum body surface temperature when their number of huddling partners increased by one or two siblings, whereas pups with heavier body mass did not show significant increases in maximum body temperature when experiencing such changes (Figure 2). This strong link between changes in the number of huddling partners and body surface temperature in lighter pups might, on the one hand, arise from a higher vulnerability of such pups due to their less favorable body surface area-to-volume ratio.

On the other hand, as lighter pups had significantly fewer neighbours than heavier ones (Figure 3) and thus typically a comparatively smaller body surface in contact with siblings, they potentially had more to gain from increasing their number of neighbours.

Most probably as a consequence of this, lighter pups – which usually had less neighbours in the huddle and thus typically occupied more peripheral positions – showed a significantly greater effort to reach the centre of the huddle by rooting and climbing over and under their littermates (Figure 4).

Conclusions

The present findings help explain why lighter pups with lower energy reserves invest more time and presumably energy than their heavier sibs in rooting between and climbing over littermates, apparently in an attempt to gain contact with at least one additional neighbour.

With our ongoing research, we are currently investigating whether such within-litter differences in sibling interactions might lead to the emergence of different and stable behavioural types during later life.

Related Literature

Bautista, A., Zepeda, J.A., Reyes, V., Féron, C., Rödel, H.G., Hudson, R. (2017) Body mass modulates huddling dynamics and body temperature profiles in rabbit pups. *Physiology & Behavior* 179, 184–190.

Rödel, H.G., Hudson, R., Bautista, A., Féron, C. (2017) Early development and the emergence of individual differences in behaviour among littermates of wild rabbit pups. *Physiology & Behavior* 173, 101–109.

Bautista, A., Zepeda, J.A., Reyes-Meza, V., Martínez-Gómez, M., Rödel, H.G., Hudson, R. (2015) Contribution of within-litter interactions to individual differences in early postnatal growth in the domestic rabbit. *Animal Behaviour* 108, 145–148.

Rödel, H.G., Bautista, A., García-Torres, E., Martínez-Gómez, M., Hudson, R. (2008) Why to be a lazy pup: milk intake and competition for the teat in the huddle and daily milk intake in domestic rabbits. *Physiology & Behavior* 95, 141–148.

ANEXO III

Zepeda A, Reyes-Meza V, Bautista A (2017) La personalidad animal. ¿Cómo ves? 219: 30-33

La personalidad animal

Por **José Alfredo Zepeda Zempoaltecatl,
Verónica Reyes Meza y Amando Bautista Ortega**

No sólo los humanos tienen personalidad. Otros animales, desde insectos hasta mamíferos, muestran diferencias de conducta individuales. Estas diferencias se deben tanto a factores genéticos como a la influencia del ambiente, la madre y los hermanos.

"¿Por qué no eres como tu hermano?", dice un padre desesperado a uno de sus hijos. Quienes no somos hijos únicos estamos expuestos a oír esto y a que nuestra conducta se compare desfavorablemente con la de algún hermano. Sin embargo, la mayoría de las veces no entendemos por qué somos diferentes y tampoco por qué nuestros padres no aceptan nuestra personalidad.

¿Ocurrirá lo mismo con otras especies animales? Si tienes perro, sabes que puedes percibir sus estados de ánimo: si está triste, alegre o enojado. También sabes que dos perros de la misma raza pueden ser muy diferentes; por ejemplo, uno agresivo y el otro amigable. Podríamos sospechar que los animales tienen

personalidad, y en el caso de perros y gatos no será demasiado descabellado. Pero seguramente sería más difícil aceptar que una mosca, una araña o un escarabajo también tienen personalidad. Pues los etólogos (biólogos que estudian el comportamiento de los animales en estado silvestre) han notado diferencias individuales en el comportamiento de estos organismos, y las llaman personalidad. Por increíble que parezca, hay más estudios sobre la personalidad en invertebrados que en mamíferos, el grupo de animales al que pertenecemos los humanos.

Síndromes conductuales

Ya desde 1872 Darwin mostró gran curiosidad por las evidentes diferencias entre animales de una misma especie y

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Podemos percibir el estado de ánimo de nuestras mascotas, sabemos cuándo están tristes, alegres o enojadas.

Fotos: Eric Isselee/Shutterstock

su aparente similitud con los humanos al expresar emociones. Esta curiosidad para entender las diferencias individuales de conducta persiste entre los etólogos, quienes han reportado que existen diferencias en conducta entre animales genéticamente cercanos del mismo sexo y edad, tanto en invertebrados como en vertebrados. Este conocimiento está condensado en el libro de Claudio Carere y Dario Maestripieri *Animal Personalities: Behavior, Physiology, and Evolution* (*Personalidades animales: comportamiento, fisiología y evolución)*, publicado por la Universidad de Chicago.

Hace más de 50 años, el etólogo Nikolaas Tinbergen, del Departamento de Zoología de la Universidad de Oxford, sentó las bases para entender la función biológica, la evolución y el origen de la personalidad animal en un artículo publicado en 1963 en la revista *Zeitschrift für Tierpsychologie* (hoy llamada *Ethology*). Tinbergen ganó el Premio Nobel de Fisiología en 1973, junto con los biólogos Karl R. Von Frisch y Konrad Lorenz, por sus descubrimientos sobre la organización y respuesta del comportamiento instintivo.

Para que una diferencia individual en conducta se considere como un rasgo de personalidad, debe ser estable a lo largo del tiempo. Por ejemplo, si un individuo es agresivo durante la etapa juvenil y sigue

Después del parto un hámster (*Mesocricetus auratus*) hembra puede devorar a sus hijos, si existen condiciones ambientales adversas. (Foto: Shutterstock).

siendo agresivo al alcanzar la madurez, entonces se dice que tiene una personalidad agresiva. El rasgo también debe ser consistente entre contextos; por ejemplo, un individuo que se arriesga a explorar un área abierta y novedosa para él, también pasará más tiempo buscando alimento. Estas diferencias de conducta individuales estables y consistentes reciben el nombre de síndromes conductuales, estilos de afrontamiento o personalidad animal.

De manera general se han descrito dos tipos de personalidad animal: individuos proactivos y reactivos. Los proactivos se caracterizan por ser más activos, alejarse más del lugar en el que nacieron y reaccionar al peligro huyendo o peleando. Los reactivos son menos activos, exploran menos y se paralizan ante el peligro.

Genética y epigenética

Sabemos que la personalidad tiene un componente genético, que nos predispone a comportarnos de cierta manera. En 1942 Calvin S. Hall, de la Universidad Western Reserve en Ohio, Estados Unidos, y su equipo, generaron dos líneas de ratas grises (*Rattus norvegicus*) con distintos niveles de agresión. Alexis Edwards, del Departamento de Genética del Centro W.M. Keck de Biología Conductual de la Universidad Estatal de Carolina del Norte, obtuvo dos linajes de moscas de la fruta (*Drosophila melanogaster*) que mostraban distintos niveles de agresión. Alexander Weiss y sus colegas en la Universidad de Tucson, Arizona, encontraron, en 2000, que el rasgo de dominancia entre chimpancés podía ser heredado. Sin embargo, hoy en día se reconoce cada vez más la importancia del ambiente para generar la variación conductual. El área de las ciencias biológicas que estudia cómo actúa el ambiente sobre la genética se denomina epigenética.

Influencia materna

En los mamíferos, la madre es uno de los factores determinantes en la fisiología y conducta de los crías desde el momento de la concepción hasta cerca del destete, cuando el individuo es capaz

Los estudios de Weiss y sus colegas encontraron que el rasgo de dominancia en los chimpancés se hereda (*Pan troglodytes*).

de alimentarse de comida sólida. Martha Weinstock, especialista en los efectos del estrés prenatal en mamíferos, encontró en roedores y primates —humanos y no humanos— que el estrés de las madres durante la gestación afecta a largo plazo la conducta y fisiología de las crías. Weinstock, de la Universidad Hebrea de Jerusalén, colocó ratas gestantes en tubos para restringirles el movimiento. Cuando las crías alcanzaron la edad adulta, mostraron neofobia (miedo a experien-

MOTIVACIONES DE LA CONDUCTA

Cuando estudiamos la conducta animal tendemos a antropomorfizarla, es decir, a explicarla como si fuera humana, lo que nos impide entender lo que en realidad significan las conductas de los animales. Por ejemplo, si vemos a una hembra de hámster (*Mesocricetus auratus*) comerse a una de sus crías después del parto, nos puede parecer una conducta aberrante, como una imagen sacada de alguna pintura de Goya. Sin embargo, los etólogos y psicobiólogos explican que esta conducta ocurre cuando la hembra percibe que las condiciones ambientales son adversas (hay poco alimento, mal clima, muchos depredadores) y el infanticidio, más allá de cualquier connotación moral, se comete en favor de la propia supervivencia.

Aunque la mayoría de los estudios de personalidad animal evalúan la osadía colocando a los individuos en un campo abierto, también hay ejemplos de otras conductas en diferentes especies.

Especie	Rasgo de personalidad	Prueba
León (*Panthera leo*)	Liderazgo	Exposición a un león intruso.
Caballos (*Equus caballus*)	Ansiedad	Separación de la madre.
Borrego cimarrón (*Ovis canadensis*)	Docilidad	Reacción ante la presencia humana.
Conejo (*Oryctolagus cunniculus*)	Respuesta ante el estrés	Reacción al chillido de otro conejo.
Vaca (*Bos taurus*)	Valentía	Reacción ante un comedero diferente.
Conejillo de indias (*Cavia aperea*)	Timidez	Salto desde la mano humana que lo sostiene.
Carbonero común (*Parus major*)	Exploración	Conducta de vuelo y salto de una rama a otra.
Serpiente (*Thamnophis terrestris*)	Temperamento defensivo	Respuesta ante una amenaza simulada.
Cerdo doméstico (*Sus scrofa*)	Ansiedad	Desplazamientos dentro de un laberinto.
Rata (*Rattus norvegicus*)	Proactividad	Exposición a un aparato que da descargas eléctricas.
Trucha arcoiris (*Oncorhynchus mykiss*)	Curiosidad	Exploración de objeto novedoso colocado en su pecera.
Pulpo (*Octopus tetricus*)	Comunicación social	Reacción ante el video de otro pulpo que cambia de color.
Araña (*Larinioides sclopetarius*)	Audacia	Conducta de exploración ante un depredador.
Abeja (*Apis mellifera*)	Toma de decisiones	Eficiencia en la elección de sitios para construir la colmena.
Hormiga (*Temnothorax longispinosus*)	Osadía	Búsqueda de alimento ante un depredador y sin él.
	Conducta maternal	Acicalamiento de las pupas (fase previa a la edad adulta).

La intensidad del cuidado maternal puede influir en el desarrollo de diferencias individuales en la conducta.

cias nuevas, medido como el tiempo que tardaron en interactuar con un objeto desconocido) y déficits en el número de interacciones con miembros de su misma especie. Según Weinstock, a pesar de que la placenta tiene mecanismos para proteger al embrión, la exposición constante de la madre a agentes causantes de estrés afecta su respuesta inmunitaria a virus infecciosos y, en consecuencia, deja vulnerables a los embriones, lo que induce efectos a largo plazo, como depresión, ansiedad y otros trastornos del estado de ánimo.

El cuidado que dan las madres a las crías también puede influir en el desarrollo de diferencias individuales de conducta. Esto lo comprobó Danielle Champagne, de la Universidad de Leiden, estudiando dos variedades de ratas: una de madres que constantemente lamían los genitales de sus crías, lo que es necesario para la excreción; y la otra de madres que lo hacían con menor frecuencia. Cuando las crías llegaron a la edad adulta, las menos lamidas se estresaron más.

El caso de los humanos no es muy diferente (véase *¿Cómo ves?* No. 210). Si una mujer embarazada sufre estrés de manera crónica, la personalidad de su bebé se verá afectada. Si a un hermano le dan más atención que a otro cuando son bebés, esto también es suficiente afectar el sistema nervioso de ambos y, por lo tanto, el desarrollo de la personalidad. Sin embargo, la conducta de la madre no es el único factor presente en el ambiente de desarrollo temprano. El comportamiento de los hermanos también es importante.

Los hermanos también influyen

Los científicos que investigan el origen de diferencias entre hermanos han reportado que el orden de nacimiento afecta la conducta de los individuos. Desde hace más de 20 años, Frank J. Sulloway, del

No todas las crías de conejos se benefician por igual de las ventajas térmicas de agruparse; los más gordos suelen estar al centro y esto traerá consecuencias sobre su crecimiento y personalidad.

En experimentos con ratas, las hembras que se desarrollaron entre dos machos durante la gestación resultan menos atractivas para los machos; son más agresivas y dominantes.

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Instituto de Investigación Social y de la Personalidad de la Universidad de Berkeley, California, ha sugerido que los primogénitos tienden a ser más responsables y obedientes, y a alcanzar mayores logros académicos que sus hermanos menores, los cuales tienden a ser creativos, afables y un poco rebeldes. Así, pese a que compartimos con nuestros hermanos la mayoría de los genes y el ambiente de desarrollo, podemos tener una personalidad muy diferente, lo cual puede deberse, en parte, a la necesidad de obtener la atención de nuestros padres.

El origen de estas diferencias individuales se ha estudiado tanto en humanos como en otras especies animales. En los mamíferos, los hermanos tienen un efecto sobre el desarrollo de la personalidad de los individuos desde etapas prenatales. El ejemplo más claro es la influencia del sexo de los hermanos en el ambiente intrauterino. En experimentos con ratas, ratones, conejos y cerdos, los fetos hembras que se desarrollaron entre dos fetos machos presentan distancias mayores

Si bien hay marmotas (*M. flaviventris*) que son osados cuando son juveniles algunas dejan de serlo al llegar a la adultez. (Foto: Shutterstock).

entre el ano y los genitales, son menos atractivas para los machos, además de más agresivas y dominantes. Esto es porque los testículos de los fetos machos secretan la hormona masculinizante testosterona alrededor del último tercio de la gestación, contaminando el ambiente de desarrollo de sus hermanos.

En nuestro grupo de investigación en el Laboratorio de Psicobiología del Desarrollo en la Universidad Autónoma de Tlaxcala, junto con la investigadora Robyn Hudson, del Instituto de Investigaciones Biomédicas de la UNAM, hemos estudiado desde hace dos décadas el efecto de los hermanos sobre el desarrollo de la personalidad en el conejo doméstico (*Oryctolagus cuniculus*). Las crías del conejo nacen poco desarrolladas, con los ojos cerrados y con poca movilidad. Como nacen sin pelo y no pueden mantener su temperatura corporal, dependen completamente de sus hermanos para obtener calor.

Durante la primera semana de vida, las crías se apiñan en el nido. Pero no todas las crías se benefician de igual manera de las ventajas térmicas de agruparse. Descubrimos que las más pesadas están la mayor parte del tiempo en el centro del agrupamiento (el sitio más ventajoso debido a su aisla-

miento térmico), lo cual tiene consecuencias sobre su crecimiento y personalidad a largo plazo. Las crías compiten fuertemente por estar en el centro, y las que permanecen la mayor parte del tiempo en la periferia (las más ligeras), en la edad adulta tienden a ser más osadas que sus hermanos que estuvieron en el centro. Este fenómeno también está reportado en el conejillo de indias (*Cavia aperea*) y en ratas.

Pero regresemos a la pregunta inicial del artículo: ¿por qué, si comparto con mis hermanos casa, padres y recursos, somos tan diferentes? Para conocer la respuesta habría que indagar lo que ocurrió durante nuestro desarrollo, específicamente cómo interactuaron los genes y el ambiente durante las etapas tempranas. A grandes rasgos, podemos decir que somos diferentes de nuestros hermanos debido a los distintos factores que interactuaron para desarrollar nuestra personalidad, pues las diferencias individuales de conducta son producto de la herencia genética, del ambiente de desarrollo temprano y de las demandas ambientales. 👁

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Verónica Reyes-Meza es neuropsicóloga y doctora en neuroetología. Actualmente es investigadora del Centro Tlaxcala de Biología de la Conducta, UATx-UNAM.

Amando Bautista se formó como psicobiólogo del desarrollo y ecólogo de la conducta. Actualmente es investigador del Centro Tlaxcala de Biología de la Conducta, UATx-UNAM.

ANEXO IV

Zepeda JA, Bautista A, Reyes-Meza V, Martínez-Gómez M, Hudson R (2018) Ontogeny of individual differences in behavior in the domestic rabbit: the role of prenatal and early postnatal life. Abstracts. Developmental Psychobiology. 2018;60:S4–S76

42. ONTOGENY OF INDIVIDUAL DIFFERENCES IN BEHAVIOR IN THE DOMESTIC RABBIT: THE ROLE OF PRENATAL AND EARLY POSTNATAL LIFE

Thu Nov 9, 2017
⊙ 5:30 PM - 7:30 PM
♀ Monticello Ballroom and National Ballroom

Description

Authors: J.A. Zepeda; A. Bautista; V. Reyes-Meza; M. Martinez Gomez; R. Hudson

42. ONTOGENY OF INDIVIDUAL DIFFERENCES IN BEHAVIOR IN THE DOMESTIC RABBIT: THE ROLE OF PRENATAL AND EARLY POSTNATAL LIFE. J.A. Zepeda1, A. Bautista2, V. Reyes-Meza2, M. Martínez Gómez2,3, R. Hudson3. 1Doctorado en Ciencias Biológicas, UATX, Tlaxcala, México. 2Centro Tlaxcala de Biología de la Conducta UATX, Tlaxcala, México. 3Instituto de Investigaciones Biomédicas UNAM, Ciudad de México, México. feyozepeda@gmail.com Consistent and stable individual differences in behavior, known as animal personality, have been studied for several decades by behavioral ecologists and psychobiologists in a wide range of taxa, from arthropodes to apes. However, studies of the ontogeny of personality are scarce, at least in vertebrates. In this work we asked if prenatal development (relative position in utero), in interaction with the first postnatal week (position within the litter huddle), drive the expression of personality traits across the preweaning period. In a first step, from postnatal days (PD) 1-7 we measured growth and position in the huddle in 36 pups from six litters. We performed two pre-weaning behavioral tests: handle-restriction (PD 2-7) and exploratory behavior (PD 17-19). We predict that central (heavier) pups within the huddle will show longer latencies to struggle and leave the start box later and explore less than their peripheral sibs, and that this will be modulated by relative position in the uterus and the sex of intrauterine neighbors. The data are currently under analysis. [CONACyT: 407512, PAPIIT IN212416].

Speaker:

Jose Alfredo Zepeda Zempoaltecatl
Ph D Student , CTBC UAT

engagement, our greatest tool for sharing research is a relatively recent discovery: the Internet. Through her experience across both academia and online engagement, Dr. Zbukvic will open a discussion on why and how researchers can harness this remarkable tool to build their impact. Dr. Zbukvic will share her tips for using Twitter, Wikipedia, and academic social networks to build your engagement across academia and beyond, and will discuss the rise of alternative metrics for measuring research impact, known as Altmetrics.

ONTOGENY OF INDIVIDUAL DIFFERENCES IN BEHAVIOR IN THE DOMESTIC RABBIT: THE ROLE OF PRENATAL AND EARLY POSTNATAL LIFE

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Consistent and stable individual differences in behavior, known as animal personality, have been studied for several decades by behavioral ecologists and psychobiologists in a wide range of taxa, from arthropodes to apes. However, studies of the ontogeny of personality are scarce, at least in vertebrates. In this work we asked if prenatal development (relative position in utero), in interaction with the first postnatal week (position within the litter huddle), drive the expression of personality traits across the preweaning period. In a first step, from postnatal days (PD) 1–7 we measured growth and position in the huddle in 36 pups from six litters. We performed two pre-weaning behavioral tests: handle-restriction (PD 2–7) and exploratory behavior (PD 17–19). We predict that central (heavier) pups within the huddle will show longer latencies to struggle and leave the start box later and explore less than their peripheral sibs, and that this will be modulated by relative position in the uterus and the sex of intrauterine neighbors. The data are currently under analysis.

LINKS BETWEEN SYMPATHETIC AUTONOMIC NERVOUS SYSTEM RESPONSIVITY DURING COGNITIVE CONTROL AND SOCIAL-EMOTIONAL OUTCOMES IN EARLY CHILDHOOD

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Despite the extensive literature on the relations between parasympathetic nervous system responses and social-emotional functioning, little work examined the role of sympathetic nervous system (SNS) responses in social-emotional functioning in early childhood. Greater SNS responsivity, as indexed by shortened pre-ejection (PEP) intervals, reflects the body's rapid excitatory (fight or flight) responses to deal with external stress. Greater SNS activation during a prolonged cognitive task may predict less adaptive social-emotional outcomes given that such responses may reflect heightened stress reactivity towards mildly stressful challenges. Our goal was to examine whether SNS responsivity during a prolonged cognitive task relates to children's social-emotional outcomes. Participants ($N = 207$) were recruited as part of a larger longitudinal study examining the integration of physiology, emotion and cognition in early childhood. PEP was measured at rest and during a Go/No-Go task (8 minutes) in kindergarten. SNS responsivity was calculated as baseline minus task PEP, such that positive values reflected SNS activation. Emotion regulation behaviors were observed in the laboratory and teachers reported on children's emotional reactivity and the quality of their relationship in kindergarten and first grade. Greater SNS responsivity during cognitive challenge in kindergarten was associated with lower levels emotion regulation behaviors ($r = -.14$-$.20$, $p < .05$), greater teacher-report of emotional reactivity ($r = .24$, $p < .05$), and lower levels of overall positive relationship with teachers ($r = -.20$, $p < .05$) one year later. These findings suggest that greater SNS responsivity during a prolonged mild-stress task may be linked with less adaptive social-emotional outcomes in early childhood.

ACKNOWLEDGMENTS