



UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
POSGRADO EN CIENCIAS BIOLÓGICAS
INSTITUTO DE INVESTIGACIONES BIOMÉDICAS

DESARROLLO DE DIFERENCIAS INDIVIDUALES EN LA CONDUCTA DEL GATO
DOMÉSTICO

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTORA EN CIENCIAS

PRESENTA:

ANDREA CITLALLI URRUTIA SCHROEDER

TUTORA PRINCIPAL DE TESIS: DRA. ROBYN ELIZABETH HUDSON

INSTITUTO DE INVESTIGACIONES BIOMÉDICAS, UNAM

COMITÉ TUTOR: DRA. LAURA ROXANA TORRES AVILÉS

INSTITUTO DE ECOLOGÍA, UNAM

DR. FRANCISCO AURELIO GALINDO MALDONADO

FACULTAD DE MEDICINA VETERINARIA Y ZOOTECNIA, UNAM

CIUDAD UNIVERSITARIA, CD. MX., 2022.



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M. en C. Ivonne Ramírez Wence
Directora General de Administración Escolar, UNAM
Presente

Me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día **05 de septiembre de 2022** se aprobó el siguiente jurado para el examen de grado de **DOCTORA EN CIENCIAS** de la estudiante **URRUTIA SCHROEDER ANDREA CITLALLI** con número de cuenta **308329113** con la tesis titulada "**Desarrollo de diferencias individuales en la conducta del gato doméstico**", realizada bajo la dirección de la **DRA. ROBYN ELIZABETH HUDSON**, quedando integrado de la siguiente manera:

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Sin otro particular, me es grato enviarle un cordial saludo.

ATENTAMENTE
"POR MI RAZA HABLARÁ EL ESPÍRITU"
Ciudad Universitaria, Cd. Mx., a 24 de octubre de 2022

COORDINADOR DEL PROGRAMA



DR. ADOLFO GERARDO NAVARRO SIGÜENZA



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Resumen

La “personalidad animal” es un campo relativamente nuevo de la conducta animal que estudia las diferencias interindividuales estables (repetibles) en la conducta. Su amplio ámbito de interés cubre tanto las causas últimas como las próximas de la personalidad, relacionando las conductas repetibles con la ecología y evolución; estudia cómo se estructuran en síndromes conductuales, y su relación con las respuestas de estrés (formando *coping styles*, estilos de afrontamiento), entre otras cuestiones. La mayoría de la investigación sobre personalidad animal hasta la fecha evalúa el comportamiento repetible en un solo punto en el tiempo o en escalas de tiempo muy cortas, y el desarrollo de las dichas diferencias ha recibido poca atención. Se puede esperar que la personalidad a lo largo de la vida sea estable debido a restricciones genéticas, anatómicas, de desarrollo y de otro tipo que limitan su flexibilidad, o podría ser inestable debido a cambios de nicho y períodos de reorganización anatómica y funcional que pueden ocurrir a lo largo del desarrollo.

Esta tesis examina las diferencias individuales estables en el comportamiento a lo largo del desarrollo en un mamífero altricial. El Capítulo 1 consiste en una revisión de la literatura sobre el tema. En los siguientes capítulos, se reporta un estudio longitudinal sobre la personalidad en el gato doméstico. Estudiamos 74 crías de gato doméstico (38 machos, 38 hembras) de 16 camadas, de seis madres diferentes, criadas en un hogar privado; al destete (2 meses de edad), las crías fueron adoptadas como mascotas y reubicadas a hogares separados. En el Capítulo 2, establecimos que existían diferencias individuales estables al destete en cinco pruebas conductuales biológicamente relevantes, que desarrollamos previamente (Anexos 1 y 2). En el Capítulo 3, dimos seguimiento a un subconjunto de los gatos del Capítulo 2 y repetimos cuatro de las pruebas conductuales a lo largo del desarrollo y hasta la edad adulta (a los 6, 12 y 18 meses de edad) para ver si las diferencias individuales persistían y si surgían síndromes conductuales. En el Capítulo 4, estudiamos los estilos de afrontamiento en los mismos individuos, evaluando la estabilidad a largo plazo de un

indicador fisiológico de estrés: el cambio de temperatura superficial de los ojos y la nariz (que se han propuesto como indicadores de hipertermia inducida por estrés) y su posible asociación con el comportamiento, incluida la vocalización, en una situación estresante.

Hallamos algunas diferencias individuales estables dentro de cada etapa de la vida estudiada, y en tres de las cuatro pruebas, la repetibilidad tendía a aumentar con la edad. Esto se atribuyó a una combinación de disminución de la varianza a nivel del individuo (los individuos se vuelven más estables) y aumento de la varianza entre individuos (los individuos se vuelven más distintos entre sí) con la edad. Además, aunque las diferencias individuales estaban presentes en todas las clases de edad y la repetibilidad era alta entre las clases de edad consecutivas, la personalidad cambió gradualmente a lo largo de las edades estudiadas. No encontramos evidencia de síndromes conductuales a ninguna edad en este conjunto de datos, en contraste con nuestros resultados anteriores en gatos adultos alojados en un refugio para animales, donde observamos un síndrome aparentemente basado en diferencias en conductas orientadas hacia los humanos (Anexo 1); sugerimos que estos resultados contrastantes pueden deberse a diferencias en los antecedentes y experiencias pasadas de los gatos. Finalmente, las medidas termográficas de los ojos y nariz no mostraron un patrón consistente de respuesta térmica a ninguna edad en una prueba estresante, lo que sugiere que, al menos en el gato doméstico, estas medidas pueden no ser indicadores confiables de estrés e hipertermia inducida por estrés. Sin embargo, observamos diferencias individuales estables en varias características de la vocalización en y entre las clases de edad, y proponemos a la vocalización como una herramienta potencialmente útil para explorar las diferencias individuales en la respuesta a situaciones estresantes y estilos de afrontamiento en el gato.

Este estudio aporta nuevos hallazgos sobre la consistencia a largo plazo de las diferencias individuales en el comportamiento, los síndromes conductuales y los estilos de afrontamiento en un mamífero altricial, desde la edad del destete hasta la edad adulta.

Abstract

“Animal personality” is a relatively new field of animal behaviour that studies stable (repeatable) inter-individual differences in behaviour. Its broad scope of interest covers both ultimate and proximate causes of personality, by relating repeatable behaviours to aspects of ecology and evolution, studying how they are structured in behavioural syndromes (also known as ‘personality types’ or ‘behavioural types’), and studying their relationship with stress responses (forming ‘coping styles’), among other questions. Most of the animal personality research to date has evaluated repeatable behaviour at a single point in time or over very short time scales, and the development of individual differences has received scant attention. Personality across the lifetime could be expected to be stable due to genetic, anatomical, developmental and other constraints which limit its flexibility, or it could be unstable due to niche shifts and periods of anatomical and functional reorganization that may occur throughout development.

This dissertation examines stable individual differences in behaviour across development in an altricial mammal. In Chapter 1, a literature review on this subject is presented. In the following chapters, we report a study conducted on personality in the domestic cat using a longitudinal approach. We studied a cohort of 74 mixed-breed domestic kittens (38 males, 38 females) from 16 litters, from six different mothers kept in a private home; at weaning (2 months of age), the kittens were adopted as pets and went to separate homes. In Chapter 2, we established that stable individual differences were present at weaning using five biologically relevant behavioural tests previously developed by our research group (Annexes 1 and 2). In Chapter 3, we followed a subset of the kittens from Chapter 2 and repeated four of the tests several times across the cats’ development and into adulthood (at 6, 12 and 18 months of age) to see if the individual differences persisted, and if behavioural syndromes emerged. In Chapter 4, we studied coping styles in the same individuals, by evaluating the long-term stability of a physiological indicator of

stress – surface temperature change of the eye and nose, which has been proposed as an indicator of stress-induced hyperthermia – and its potential association with behaviour, including vocalization, in a stressful situation.

We found at least some stable individual differences within every life stage studied, and in three of the four tests, that within-age repeatability tended to increase with age. This was attributable to a combination of within-individual variance declining (individuals becoming more stable) and between-individual variance increasing (individuals becoming more distinct from each other) with age. Additionally, although individual differences were present in every age class, and repeatability was high between consecutive age classes, personality gradually changed over the full age span of the study. We did not find evidence of behavioural syndromes at any age in this dataset, in contrast to previous results on adult cats housed in a shelter environment where we observed a syndrome apparently based on differences in human-oriented behaviours (Annex 1); we suggest that these contrasting results may be due to differences in the cats' background and past experiences. Finally, thermographic measures of eye and nose temperature showed no consistent patterning of thermal response at any age in a stressful test, suggesting that –at least in the domestic cat – these measures may not be reliable indicators of stress and stress-induced hyperthermia. However, we did observe stable individual differences in various characteristics of vocalization within and across age classes, and we propose vocalization as a potentially useful tool for exploring individual differences in responding to stressful situations and in coping styles in the cat.

This study contributes novel findings on the long-term consistency of individual differences in behaviour, behavioural syndromes and coping style in an altricial mammal, from weaning age to adult life.

Introducción general

La personalidad animal se define como las diferencias individuales en el comportamiento que son estables a lo largo del tiempo y/o en diferentes contextos (Gosling, 2001; Sih, Bell, Johnson y Ziemba, 2004). En las últimas décadas, se ha buscado comprender los impactos y las aplicaciones de la personalidad animal en campos tan diversos como la ganadería (p. ej., Hedlund y Løvlie, 2015), el bienestar (p. ej., Tetley y O'Hara, 2012), la conservación (p. ej., Mcdougall et al., 2004; Powell y Gartner, 2011), el control de plagas (p. ej., Morales et al., 2013), el ecoturismo (p. ej., Griffin et al., 2017), la urbanización (p. ej., Lapiedra et al., 2017) y la respuesta de los animales al cambio climático (Cockrem, 2013).

Existen varias hipótesis (no mutuamente excluyentes) que pueden explicar la existencia y evolución de la personalidad animal: como comportamiento dependiente del estado de los individuos o de su ambiente (McElreath y Strimling, 2006; Wolf y Weissing, 2010), compromisos (*trade-offs*) de historia de vida (Wolf et al., 2007; Réale et al., 2010), selección dependiente de la frecuencia que da como resultado estrategias evolutivamente estables (Maynard Smith, 1982; Dall et al., 2004), selección fluctuante (Kight et al., s.f.) y por equilibrio mutación-selección (Zhang y Colina, 2005). La variación individual en el comportamiento se puede atribuir a una variedad de mecanismos subyacentes que operan a varios niveles, como efectos genéticos (van Oers y Sinn, 2013), neuroendocrinos (Caramaschi et al., 2013), ambientales (Carere et al., 2005) y de desarrollo (Duckworth, 2010). Estas causas próximas y últimas de la personalidad se examinan con más detalle en el Capítulo 1.

No solo los rasgos individuales pueden mostrar consistencia o repetibilidad, sino que dos o más comportamientos pueden correlacionarse consistentemente entre individuos, formando un “síndrome conductual” (Sih et al., 2004). Esto podría ser una correlación entre la expresión del

mismo tipo de comportamiento en dos contextos diferentes; por ejemplo, un síndrome de agresión, donde los individuos que son muy agresivos en un contexto también lo son en otro. Alternativamente, un síndrome conductual puede referirse al acoplamiento de dos (o más) comportamientos distintos pero correlacionados; por ejemplo, si los individuos más agresivos también son más activos. Los síndromes conductuales podrían ser producto de la selección natural si ciertas combinaciones de comportamientos se ven favorecidas por selección correlacional (Bell, 2007), o podrían ser un producto secundario por integración funcional (hormonal, neuronal, metabólica) o genética, en donde la expresión de varias conductas es afectada por los mismos mecanismos (Duckworth, 2010).

Por otro lado, los “estilos de afrontamiento” abarcan las respuestas al estrés en las que existe consistencia individual a lo largo del tiempo (Koolhaas et al., 1999). Esta respuesta al estrés engloba un conjunto de respuestas conductuales y fisiológicas; las respuestas conductuales dependerán de la especie y del estresor al que se enfrenta, pero incluyen conductas de huida, vigilancia y comunicación (p. ej. vocalización; Rushen, 2000). Por otro lado, durante la respuesta al estrés en vertebrados, el sistema nervioso simpático y el eje hipotálamo-pituitario-adrenal (HPA) liberan la hormona catecolamina epinefrina de la médula adrenal y hormonas glucocorticoides de la corteza adrenal, que tienen una amplia gama de efectos en el cuerpo (Romero y Butler, 2008) que preparan a los organismos para respuestas de “lucha o huida” (*fight or flight*). Aunque los mecanismos y la regulación de la respuesta al estrés son compartidos por una especie, existen diferencias consistentes entre los individuos en la magnitud de sus respuestas al estrés que se han denominado "estilos de afrontamiento" (Koolhaas et al., 1999). En general, se han descrito dos tipos principales de respuestas: los individuos “proactivos” se caracterizan por una alta reactividad simpática, baja reactividad del eje HPA y mayor audacia y agresión, mientras que los individuos “reactivos” se caracterizan por una baja reactividad simpática, alta reactividad del eje HPA, y

menor audacia y agresión (Korte et al., 2005). Finalmente, el estrés prolongado o crónico puede tener efectos adversos graves sobre el sistema inmune, el metabolismo, la reproducción y la cognición (Sapolsky et al., 2000), por lo que las consecuencias de los estilos de afrontamiento son importantes no solo desde una perspectiva ecológica y evolutiva, sino también para fines aplicados a la salud y el bienestar de los animales humanos y no humanos.

La mayor parte de la investigación en el campo de la personalidad animal se ha realizado en animales adultos, y una proporción aún mayor es transversal, es decir, la personalidad se evalúa en un solo punto en el tiempo o en escalas de tiempo muy cortas (Bell et al., 2009). Sin embargo, los animales juveniles y adultos se enfrentan a diferentes entornos internos (perceptuales, fisiológicos) y externos (ecológicos, sociales), y pueden enfrentar diferentes presiones selectivas, por lo que es importante estudiar la personalidad y cómo varía entre diferentes clases de edad y a lo largo del desarrollo. Se pueden formular dos hipótesis opuestas con respecto a la consistencia de la personalidad a lo largo de la vida. Por un lado, la personalidad puede cambiar entre etapas de vida debido a los cambios de nicho y los cambios perceptivos, endocrinos, neurales y morfológicos que experimentan los animales durante el desarrollo, lo que podría alterar patrones anteriores de personalidad y posiblemente desvincular síndromes conductuales entre etapas de vida. Por otro lado, la variación en el comportamiento está basada en componentes físicos y fisiológicos internos, y la velocidad y la medida en que éstos pueden cambiar están limitadas por múltiples restricciones funcionales físicas y de desarrollo, que potencialmente también limitan la flexibilidad del comportamiento con el tiempo. Estas ideas se exploran más a fondo en el Capítulo 1.

El gato doméstico (*Felis silvestris catus*)

El gato doméstico (*Felis silvestris catus*, también conocido como *Felis catus*; Kitchener et al., 2017) es una especie carnívora, generalmente considerada facultativamente social (Turner, 2017). Al ser una de las mascotas más comunes (Driscoll, Macdonald, et al., 2009), se distribuye en todo el mundo. El ancestro más probable del gato doméstico es el gato montés africano *Felis silvestris libyca*, de acuerdo con la evidencia morfométrica, genética y arqueológica (Driscoll, Clutton-Brock, et al., 2009; Ottoni et al., 2017). A pesar de ser aparentemente candidatos pobres para la domesticación debido a su estilo de vida solitario y territorialidad (Ottoni et al., 2017), se cree que la domesticación de gatos silvestres ocurrió de una manera "autoselectiva", donde los gatos se asociaron con asentamientos humanos, y fueron tolerados por los humanos como ratoneros inofensivos (Driscoll, Clutton-Brock, et al., 2009).

Genéticamente, los gatos domésticos siguen siendo muy similares a los gatos monteses (Montague et al., 2014). La domesticación solo ha alterado ligeramente sus características morfológicas y fisiológicas (Ottoni et al., 2017): los gatos domésticos tienen patas ligeramente más cortas, un cerebro más pequeño y un intestino más largo que sus parientes más cercanos, pero conservan en gran medida el plan corporal de los gatos monteses (Driscoll, Clutton-Brock, et al., 2009). Mientras que los gatos monteses son solitarios y territoriales, los gatos domésticos adaptan su uso del espacio y estructura social al área que ocupan y la configuración de sus recursos alimentarios. En hábitats con recursos alimentarios dispersos (áreas naturales, áreas rurales), los gatos domésticos viven en bajas densidades, de manera solitaria y con ámbitos hogareños que tienden a no superponerse, mientras que alrededor de fuentes de alimentos concentrados (por ejemplo, en áreas urbanas), los gatos viven en densidades más altas, tienen ámbitos hogareños superpuestos y forman grupos llamados colonias (Normand et al., 2019).

Si bien el gato es doméstico, no es una especie *altamente* domesticada, como lo demuestran las poblaciones de gatos ferales en todo el mundo que sobreviven sin intervención humana, y por las diferencias morfológicas y de comportamiento relativamente pequeñas entre él y sus ancestros silvestres. Además, los gatos domésticos que no reciben manejo temprano por parte de humanos durante las primeras 9 semanas de vida raramente tolerarán el contacto cercano con humanos, y mostrarán comportamientos diferentes en comparación con gatos que fueron manipulados al ser crías (Turner, 2017). Por ejemplo, los gatos domésticos ferales difieren significativamente en su comportamiento vocal (y en las características acústicas de sus vocalizaciones) en comparación con los gatos domésticos mantenidos como animales de compañía, cuando se encuentran en una situación estresante (Yeon et al., 2011).

Durante décadas, el gato doméstico ha sido una especie modelo popular para estudios biomédicos (por ejemplo, de anatomía, fisiología, neurobiología, enfermedades, etc.), a veces considerado como una alternativa más económica y conveniente a los primates. Sin embargo, la investigación sobre el comportamiento de los gatos fue escasa hasta hace relativamente poco tiempo: la primera revisión científica publicada en inglés sobre el tema fue la primera edición de *The Domestic Cat: The Biology of its Behavior* en 1988. Pero el gato ofrece algunas ventajas como modelo para estudios conductuales. Bajo condiciones seminaturales, es fácil de mantener y de manejar en experimentos conductuales; y las madres permiten que personas conocidas manipulen incluso a las crías recién nacidas (Hudson et al., 2009). Además, existe un interés creciente, tanto en la ciencia experimental básica como en áreas aplicadas como el bienestar, en conocer más sobre la personalidad en el gato domesticado (Vitale Shreve y Udell, 2015).

El desarrollo del gato

Las hembras alcanzan la madurez sexual entre los 7 y 12 meses de edad, y los machos entre los 9 y 12 meses (Bateson, 2000). El cuidado parental en esta especie es exclusivamente materno, y puede existir paternidad múltiple dentro de una misma camada (Say et al., 1999). Después de un período de gestación de dos meses, las hembras paren camadas compuestas por un promedio de 4 crías (Root et al., 1995), aunque pueden tener de 1 a 9 crías (observación propia).

El gato es una especie altricial; las crías nacen con los ojos y los canales auditivos cerrados, control motor limitado, e incapacidad de termorregulación propia (Villablanca y Olmstead, 1979; Levine et al., 1980). La actividad locomotora aumenta notablemente durante la cuarta y quinta semana posnatal, junto con un mayor desarrollo de los sistemas visual y auditivo (Levine et al., 1980). En este momento las crías también comienzan a comer alimentos sólidos que, acompañado por una reducción de la lactancia, marca el inicio del período de destete (Martin, 1986; Bateson, 2000). Comienzan a dejar el nido por períodos cortos, y para la sexta semana realizan todos los movimientos de la locomoción adulta (Peters, 1983). El destete se puede considerar completo alrededor de la séptima semana de vida (Bateson, 2000), aunque depende de su hábitat que la dispersión de las crías ocurra en este periodo o algunas semanas más tarde.

Los gatos domésticos pueden vivir más de 15 años en condiciones óptimas (generalmente como animales de compañía), pero la mayoría de los gatos que viven en exteriores no sobreviven hasta esta edad. En vida libre, la tasa de mortalidad de las crías es alta debido a la depredación, enfermedades y accidentes vehiculares (en el caso de áreas suburbanas y urbanas). Por ejemplo, Izawa & Ono (1986) reportaron una tasa de mortalidad del 90.5% a los 10 meses de edad en una isla rural, y Nutter et al. (2004) reportaron una tasa de mortalidad del 75% a la edad de 6 meses en dos áreas suburbanas. Debido a esta alta tasa de mortalidad, la etapa juvenil es un período

importante para el desarrollo físico y conductual, durante el cual las crías deben aprender rápidamente para sobrevivir hasta la edad reproductiva.

Trabajos previos sobre diferencias individuales en la conducta del gato doméstico

El amplio repertorio conductual del gato doméstico lo convierte en un buen modelo para el estudio de la personalidad animal. Algunas investigaciones en este ámbito ya se han realizado utilizando una variedad de métodos (observación, encuestas a propietarios, experimentación), pero principalmente en animales adultos (ver revisión por Gartner y Weiss, 2013; Gartner, 2015; Mendl y Harcourt, 2000; Travník et al., 2020). Los gatos domésticos adultos muestran diferencias interindividuales estables a corto plazo en una variedad de conductas, como la vocalización y la actividad motora durante un confinamiento breve, su interacción con presas, y su reacción ante humanos conocidos y desconocidos (Urrutia et al., 2019; Martínez-Byer et al., 2020; Chacha et al., 2020; ver Anexos 1 y 2), conductas dirigidas hacia objetos y animales novedosos (Durr y Smith, 1997), y en su comportamiento después de la alimentación (Bradshaw y Cook, 1996). En crías, este tipo de investigaciones son escasas, aunque crías muy jóvenes (menores a 1 mes de edad) ya muestran diferencias individuales consistentes en la cantidad de locomoción y el número de vocalizaciones emitidas durante períodos repetidos de aislamiento breve (Hudson et al., 2015, 2017). Estas diferencias estables entre individuos en la cantidad de locomoción también están presentes en el entorno del nido (Raihani et al., 2014).

Sólo existen dos estudios sobre la estabilidad de la personalidad a lo largo del desarrollo en el gato. Turner et al. (1986) reportó que las clasificaciones realizadas por observadores independientes de 22 crías según su reacción general hacia los humanos (qué tan “amistosos” eran) eran estables entre las edades de 3 y 8 meses. Además, Lowe y Bradshaw (2001) encontraron que los individuos que recibían más manipulación por experimentadores cuando eran crías

reaccionaron de manera más “audaz” hacia los humanos a las edades de 4 meses y 1 año, pero este efecto ya no se observó a los 2 años de edad. Estos estudios parecen indicar que algunos comportamientos orientados hacia los humanos pueden tener cierto grado de consistencia a largo plazo, aunque no se evaluó la consistencia dentro de los grupos de edad estudiados (i.e. a corto plazo), y el desarrollo de rasgos de personalidad no relacionados con humanos sigue sin conocerse.

Con respecto a los síndromes conductuales en el gato, en algunos estudios se ha propuesto la organización de su conducta en distintas "dimensiones" de personalidad, por ejemplo “amabilidad” y “agresividad” (hacia humanos y otros gatos) (ver revisión de Travník et al., 2020). Sin embargo, conocemos sólo un estudio que utilizó pruebas conductuales repetidas para primero establecer la existencia de conductas repetibles antes de explorar la organización de las mismas en síndromes conductuales: en un estudio realizado por nuestro grupo, hallamos que gatos adultos de un refugio para animales muestran diferencias individuales consistentes en una serie de pruebas conductuales, y que dichas conductas se correlacionan entre sí para formar un síndrome conductual, al parecer basado principalmente en diferencias individuales en la reacción hacia humanos (Martínez- Byer et al., 2020; Anexo 1).

Objetivos

El objetivo principal de este estudio longitudinal es describir el desarrollo de las diferencias individuales en el comportamiento del gato doméstico en una serie de situaciones biológicamente relevantes, desde el periodo de edad pre-destete hasta la etapa adulta, y explorar la posibilidad de que los comportamientos repetibles se asocien para formar síndromes conductuales. Con este fin, primero establecimos que las diferencias individuales repetibles en el comportamiento se presentan en crías de gato doméstico en etapa pre-destete (Capítulo 2), utilizando pruebas conductuales previamente establecidas por nuestro grupo de estudio (Anexos

1, 2). A continuación examinamos la consistencia de las diferencias individuales en el comportamiento en un subconjunto de los mismos individuos, repitiendo las mismas pruebas en varias etapas de desarrollo y en la edad adulta (Capítulo 3). Finalmente, examinamos si en estos mismos individuos había evidencia de estilos de afrontamiento: diferencias individuales estables en las respuestas conductuales y fisiológicas a una situación estresante a lo largo del desarrollo (Capítulo 4).

Capítulo I

Revisión crítica del estudio del desarrollo de la personalidad animal y sus métodos

A critical review of the study of development of animal personality and its methods

Manuscrito

A critical review of the study of development of animal personality and its methods
Revisión crítica sobre el desarrollo de personalidad animal y los métodos de estudio

Introduction

Even before the publication of Darwin's *On the Origin of Species* in 1859, natural scientists recognized that variation between individuals of the same species underpins evolution (Crews, 2013). The existence of differences in behaviour between individuals is evident to anyone who observes or interacts with animals on a regular basis. What is perhaps surprising, though, is that these inter-individual differences can be stable over time, despite it being the very plasticity of behavior which enables animals to respond rapidly and adaptively to changes in environmental conditions.

Over the last two decades, thousands of theoretical and empirical research papers have been published on the subject of stable inter-individual differences in behaviour, now consolidated into a field known as "animal personality". This field's scope of interest has expanded from showing that personality in animals exists to addressing its structure and its ultimate and proximate causes, by relating it to aspects of ecology and evolution (such as differential survival rates and reproductive success; Smith & Blumstein, 2008), studying how it is structured in 'behavioural syndromes'/'personality types'/'behavioural types' (Sih et al., 2004), studying its relationship with stress response, forming 'coping styles' (Koolhaas et al., 2007), and the underlying mechanisms leading to stable differences in behaviour, e.g., genetic (van Oers & Sinn, 2013), neuroendocrine (Caramaschi et al., 2013), environmental (Carere et al., 2005), developmental (Duckworth, 2010). Furthermore, researchers are seeking to understand the effects and applications of animal personality in fields as diverse as cognition (Carere & Locurto, 2011), animal husbandry (Hedlund & Løvlie, 2015), welfare (Tetley & O'Hara, 2012), conservation (Mcdougall et al., 2004; Powell

& Gartner, 2011), pest control (Morales et al., 2013), ecotourism (Griffin et al., 2017), urbanization (Lapiedra et al., 2017) and climate change (Cockrem, 2013).

What is personality?

The rapid expansion of the field of animal personality has been accompanied by a variety of approaches to its study and the terminology used to describe it. Overall, two main definitions of animal personality (henceforth “personality”) can be identified. On one hand, the “broad-sense” definition of personality refers to consistent differences between individuals in any behaviour, though generally one that is likely to have ecological consequences (Réale et al., 2010a; Roche et al., 2016). Note that “consistency” here means that differences between individuals are largely maintained, even if mean trait values change (e.g. with age or across conditions; Réale et al., 2007). Some have pointed out that when used in the broad sense, the term “personality” could be replaced by the more descriptive “repeatable behaviour” (Roche et al., 2016). A second, “narrow-sense” definition of personality refers to consistent individual differences in suites of specific behaviours (Réale, et al., 2010a) which are then used as proxies for latent personality traits (generally those included in the 5-factor model of animal personality—see below). The narrow-sense definition of personality is restricted to behaviours generally measured in standardized tests (e.g. open-field test, novel object test, mirror test, emergence test), typically carried out in a novel or challenging context.

The 5-factor model of animal personality (Réale, et al., 2010a, 2007) is derived from psychological research on human personality, and proposes that inter-individual behavioral variation occurs on five main axes: aggressiveness (agonistic reactions to conspecifics), sociability (reactions to absence/presence of conspecifics, excluding agonistic behaviour), exploration (or neophobia; reactions to new stimuli or environments), boldness (reactions to risky situations or

stimuli), and activity (a general level of the individual's activity). This model, a simplified classification of behaviour, was presented by Réale et al. (2007) originally as a working tool for the study of personality. It has the advantages of facilitating the comparison between studies, especially in different species, and the exploration of relationships between personality traits and other variables (e.g. life-history traits, physiology, etc.). However, the 5-factor model may not be the best representation of behaviour in any given species, and may not be deeply conserved (Bell, 2017). Furthermore, there are species which show repeatable intraspecific variation in all sorts of behaviors, some of which do not fit neatly into one of these five factors – hence the usefulness of the broad-sense definition of personality.

Next to these two definitions of personality is the related concept of behavioural syndromes (Sih et al., 2004). These syndromes are behavioural correlations, usually at the population level, and can refer to either one of two cases. First, to a correlation between the expression of the same behaviour in two different environmental contexts, for example, an aggression syndrome, where certain individual funnel web spiders show higher attack tendencies on both prey and conspecifics who invade their territory (Riechert & Hedrick, 1993). Second, a behavioural syndrome can refer to the coupling of two (or more) distinct but correlated behaviours; for example, if more aggressive individuals are also more active. To investigate behavioural syndromes, researchers could use either personality traits in the broad sense or in the narrow sense. Again, the narrow-sense personality approach facilitates comparisons between species, like in the case of the boldness-aggression syndrome. This syndrome, in which bolder individuals (those more prone to risk-taking) are also more aggressive with conspecifics, is present in a variety of species, including insects (Chapman et al., 2011), fish (Huntingford & Adams, 2005), birds (Groothuis & Carere, 2005) and mammals (Malmkvist & Hansen, 2002), although it may not be universal (Wolf et al., 2007).

Why does personality exist?

At the ultimate level

There are several non-competing explanations for why personality in animals evolved. One hypothesis posits that consistent inter-individual differences in behaviour are linked to differences in state. Here, state is defined as in life-history and evolutionary game theory, and refers to any feature of the animal itself or of the environment that should be taken into account for decisions affecting fitness (e.g. body size, condition, territory size, mate quality, level of experience; Wolf & Weissing, 2010). An organism's state at any given time will influence the costs and benefits of any action it could potentially perform; for example, for an individual with low energy reserves the benefit of obtaining food could outweigh the costs of exposing itself to predators while foraging, whereas this may not be the case for an individual in better condition. If states are slow to change, then behavioural consistency can be expected (Dall et al., 2004). An extension to the idea of state-dependent personality is a model put forward by McElreath & Strimling (2006), which suggests that if individuals vary in state *and* if information about the world is noisy (i.e. the cues used by animals do not predict the environment perfectly), then selection may favour individuals in some states ignoring cues about context and instead behaving in a consistent manner, i.e. showing personality. For example, in some species a conspecific may either copulate or cannibalize a potential mate, and sometimes it is unclear which will happen (i.e. the cues are noisy). If individuals' states affect the expected costs, benefits and probabilities of the two possible outcomes (e.g. if large individuals are unlikely to be consumed), then consistent across-context differences could arise in going forward with the interaction or not (McElreath & Strimling, 2006).

A second hypothesis explains the evolution of personality by linking it to life-history trade-offs. Many of the personality traits studied in animals can be thought of in terms of risk-taking behaviour: aggression against conspecifics, boldness towards predators, exploration of a novel

environment which could expose one to unknown risks. According to life-history theory, individuals should adjust their risk-taking behaviour according to their expected future fitness (residual reproductive value). The higher an individual's expected future fitness, the more it stands to lose by exposing itself to potential danger, and therefore should be more risk-averse than an individual with lower expected future fitness. Wolf et al. (2007) developed a model showing that the existence of a trade-off between current and future reproduction would lead to polymorphic populations, in which individuals pursue different strategies according to their remaining reproductive prospects. They then demonstrated that this variation in life-history strategies selects for systematic differences in risk-aversion, which would extend to various risky situations and be stable over short time scales, thereby giving rise to animal personalities (Wolf et al., 2007). If life-history strategies include a behavioural component (risk-taking), then individuals pursuing differing life-history strategies should show consistent, long-term differences in personality associated with other relevant traits, e.g. timing of reproduction, metabolism, immune response, etc. (Réale et al., 2010b).

A third hypothesis for the evolutionary basis of personality relies on frequency-dependent selection. In evolutionary game theory, the frequencies at which competing strategies are played affect the fitness payoffs of each strategy; many game theory scenarios lead to the stable coexistence of different strategies, or behavioural types, in populations (evolutionarily stable strategies; Maynard Smith, 1982). There are two ways in which the mixture of tactics in a population can be maintained by frequency-dependent selection: either each individual is flexible and can adopt all of the strategies/behavioural types with fixed probabilities which would maintain the relative frequencies overall at the population level, or a fixed proportion of individuals of the population can play each strategy consistently. This second possibility, which corresponds to differing personalities, could evolve if individuals had information about their opponents' previous

interactions. This would select for within-individual consistency, because individuals would benefit by being predictable, by avoiding costly escalations or unfavorable interactions (Dall et al., 2004).

A fourth hypothesis involves fluctuating selection pressures caused by environmental variability, since environmental conditions (e.g. food availability, density of predators, etc.) can vary in space and time, resulting in differing environmental pressures. Although environmental heterogeneity could be expected to lead to phenotypic plasticity at the individual level (where individuals adjust their behaviour to the particular environment or microenvironment they face at any given moment) rather than the coexistence of specialized personalities (Kight et al., n.d.), if plasticity is costly then in some cases personality variation could be favoured instead. For example, in Siberian chipmunks (*Tamias sibiricus*) bolder individuals (those more likely to be trapped), had higher reproductive success in years with low food availability, whereas the reverse was true in years with high food availability (Le Cœur et al., 2015). This indicates that in the studied population, temporally fluctuating selection likely helps maintain variation in boldness.

A fifth hypothesis explains the existence of personality through mutation-selection balance. Since personality has genetic bases (as discussed in the following section), if indeed there is an optimal value for a particular behavioural trait but natural selection for it is weak (e.g. if the optimal value only provides small fitness advantages, or selection is weak due to pleiotropy), then variation in personality in the population will be maintained or recovered by mutation (Zhang & Hill, 2005). In this case, we could expect that personality differences are generated by chance. However, the non-random distribution of personality traits and the findings that many personality traits do not have a single, optimal value that maximizes fitness indicate that mutation-selection balance is not sufficient explanation for the existence of personality (Kight et al., n.d; Smith & Blumstein, 2008).

As for behavioural syndromes, they could be the product of selection if certain combinations of behaviours are favoured by correlational selection (Bell, 2007). Alternatively, they could be a side-product of constraints on behaviour, such as functional constraints or pleiotropy. An interesting aspect of behavioural syndromes is that if they result from constraints, this offers an explanation for behaviours which appear to be maladaptive in some ecological contexts (Sih et al., 2004). For example, a study found that juvenile European rabbits which were more explorative near their burrows in a field enclosure were also more aggressive and struggled sooner when handled by experimenters. Yet these highly explorative, aggressive and struggle-prone juveniles had a lower probability of survival in this life stage, possibly because their high exploration left them more exposed to predators. While this would seem to indicate that highly exploratory behaviour is maladaptive in this species, high exploration as part of a syndrome with other behaviours (such as a proclivity to struggle more or faster when caught by a predator) could constitute an adequate strategy, particularly if the syndrome is consistent over long periods of time and is advantageous in later life stages or in different environmental conditions (Rödel et al., 2014). Therefore, correlations among behaviours could impose a limit on the optimality of individual behaviours through developmental (as well as evolutionary) time (Bell, 2007; Sih et al., 2004; Wolf et al., 2007). The first four models described above explaining the evolution of personality (state-dependent behaviour, life-history trade-offs, frequency-dependent selection and fluctuating selection) have in common that consistent inter-individual behavioural differences arise as an adaptation, not a side-effect of any constraint. And yet, intrinsic constraints to behavioral flexibility likely impact the evolution of personality traits, as discussed in relation to behavioural syndromes (Sih et al., 2004). Common genetic or functional bases across personality traits can cause a correlated response to selection on non-target traits, therefore personality traits could evolve together as packages (Bell, 2007).

At the proximate level

At the proximate level, personality has been found to have a genetic basis (van Oers & Mueller, 2010); for example, up to 5% of the variation in exploration behaviour of a wild population of great tits (*Parus major*) can be explained by polymorphism of a single dopamine receptor gene (Korsten et al., 2010). The average heritability (the relative amount of genetic variation in relation to the phenotypic variation) of personality traits was calculated in a meta-analysis to be 0.26, with an average heritability of 0.36 in wild animal populations and of 0.24 in domestic and laboratory species (van Oers & Sinn, 2013). This difference is probably due to domestic and laboratory species living in more uniform, stable and benign environments where selection is largely artificial, and therefore unlikely to operate in the same manner as it would in wild populations. Moreover, genetic variation itself in domestic species may have been reduced through artificial directional selection, which could then have the effect of lowering overall personality variation (van Oers & Sinn, 2013).

Interestingly, there have been reports of personality even among genetically identical individuals of several species (sea anemone, *Anthopleura elegantissima*: Ayre & Grosberg, 1995; pea aphid, *Acyrtosiphon pisum*: Schuett et al., 2011; rose aphid, *Macrosiphum rosae*: Dehcheshmeh & Tabadkani, 2017; *Daphnia magna*: Heuschele et al., 2017; mourning gecko, *Lepidodactylus lugubris*: Sakai, 2018; pig, *Sus scrofa domesticus*: Archer et al., 2003). In these cases, the presence of individual differences could be attributed entirely to differing external conditions. Individual differences in environment and experience could create positive feedback mechanisms which fix individuals on different developmental trajectories and thereby lead to and reinforce differences in personality (Bierbach et al., 2017; Stamps & Groothuis, 2010). If this were the case, it would stand to reason that genetically identical animals experiencing the same

environmental conditions should develop, in theory, identical behavioral traits. To test this idea, Schuett et al. (2011) examined clonal pea aphids' (*Acyrtosiphon pisum*) response to an encounter with a predator (ladybird) on a leaf, and found that individuals tended to consistently either drop off the leaf as a defense mechanism or not drop off. However, small differences in rearing conditions could have affected the outcome of the study, as could the behaviour of the live ladybird in the predatory encounters. Perhaps even these small differences in experience and environment could be enough to create feedback loops affecting personality. A more recent study by Bierbach et al. (2017) tested for this even more rigorously by raising genetically identical fish (the clonal Amazon molly, *Poecilia formosa*) isolated directly after birth into rearing conditions which were as near to identical as possible, explicitly in order to dampen individuality. In contrast to their predictions, the authors found that substantial individual variation in behaviour emerged among the individuals (total distance swam in an open field test). Of course, despite even the most rigorously controlled experimental efforts, different individuals will never experience completely identical environments, and perhaps environmental differences much more minute than suspected could create potential positive feedback loops leading to personality as discussed above. But if this is indeed a mechanism for the development of individuality in otherwise 'identical' animals, it raises the question of whether the emergence and patterning of individuality is, therefore, an inevitable and potentially unpredictable outcome of development (Bierbach et al., 2017). Perhaps in these cases the personality differences in genetically and environmentally "identical" individuals are due to epigenetic variation, either stochastic or environmentally induced (Bierbach et al., 2017).

Experience and environment, including prenatal maternal effects, can impact personality throughout the lifetime. For example, the levels of hormones deposited by female birds in egg clutches are affected by environmental and maternal factors including social density, maternal age,

food availability and mate attractivity (Groothuis et al., 2005). These differing levels of steroid hormones in the egg affect offspring aggressiveness, boldness and exploratory behavior (Groothuis et al., 2005). In multiparous mammals, fetuses are affected in utero by the testosterone produced by adjacent male littermates; in domestic rabbits (*Oryctolagus cuniculus*), female rabbits that had more male neighbours performed more chin marking behaviours, which are associated with maintenance of social rank (Bánszegi et al., 2009). Postnatally, personality can be affected by a diverse set of environmental factors, including resource quality and quantity, competition, predation risk and social group size, among others (Groothuis & Taborsky, 2015). For example, in southern field crickets (*Gryllus bimaculatus*), males raised on high-protein diets were more aggressive than conspecifics raised on high-carbohydrate diets, perhaps because of their comparatively larger body size and decreased mortality; dietary differences would thus lead to state-dependent personalities (Han & Dingemans, 2017).

Individual differences in behavior have been associated with variation in endocrine (re)activity; most studies focus on hormones of the hypothalamic–pituitary–adrenal (HPA) axis, which plays an important role in stress response. For example, in great tits, two lines were artificially selected from wild populations: the FAST line is quick to explore novel environments and approach novel objects, while the opposite is true of the SLOW line (Groothuis & Carere, 2005). Interestingly, the physiological response to stress is different in the two lines even though this was not selected for directly: the SLOW line has higher HPA activation and higher body temperature in reaction to stress (Carere et al., 2003; Carere & Van Oers, 2004). While it is unclear whether endocrine differences are a cause or consequence of personality, there is an association between the two (Koolhaas et al., 2010).

As for the link between neurology and personality, individual differences in brain structure have been linked to behavioural variation as well. For example, in the American mink (*Neovison*

vision), the number of neurons in the basolateral amygdala (an important mediator of the response to aversive stimuli) was positively correlated with boldness (Wiese et al., 2018); in the brook char (*Salvelinus fontinalis*), the volume of the telencephalon (a brain region involved in the regulation of movement in space in fishes) was correlated with individuals' consistent foraging style (active or sedentary; Wilson & McLaughlin, 2010).

Development of personality

Most of the research in the field of animal personality has been conducted on adult animals, and an even greater proportion of it is cross-sectional, i.e. personality is evaluated at a single point in time or over very short time scales (Bell et al., 2009). However, the study of personality in early life stages is of interest also, since mortality rates in early life are high and a large proportion of animals do not reach maturity— meaning they face intense selective pressure (Sibly, Collett, Promislow, Peacock, & Harvey, 1997). Therefore, if personality is adaptive, then it is reasonable to expect that marked behavioural differences among conspecifics may appear early in life, and indeed there is evidence of personality in young animals of a variety of taxa (arthropods, e.g. Gyuris et al., 2012; fish, e.g. Polverino et al., 2016; amphibians, e.g. Urszán et al., 2015; reptiles, e.g. Brodie, 1993; birds e.g. McCowan & Griffith, 2014; mammals, e.g. Hudson et al., 2017).

Knowing that personality differences are present early in life, a further question is to what degree personality is stable across development. Two opposing hypotheses can be made regarding the consistency of personality across the lifetime. The first is that behaviour is unlikely to be consistent between life stages because selective pressures on juveniles often differ from those faced by adults, so dramatic niche shifts during development may result in selection altering earlier patterns of personality and possibly decoupling syndromes between stages (Sih et al., 2004). At a mechanistic level, the perceptual, endocrine, neural and morphological changes that animals

experience during development and maturation could 'override' earlier differences in behaviour, removing effects of previous juvenile experience and 'resetting' the phenotype-determining processes in adults (Brodin, 2009; Monceau et al., 2017).

However, there is also a case to be made for expecting behavioural consistency across development. All behavioural variation is underlain by internal physical components: differences in brain anatomy, neuronal connectivity, neurotransmitter synthesis and degradation, hormone secretion patterns, hormone receptor distribution, and endocrine gland morphology (e.g. size) and function (Duckworth, 2010). The speed and extent to which these components can change is limited by multiple constraints, such as the slow rate at which new neural and endocrine tissue can be built, which, depending on the species, may take from weeks to years (Duckworth, 2015), and the energetic cost of reorganizing physiological and neurological pathways (Laughlin & Sejnowski, 2003). In addition, the close integration of the neuroendocrine system can lead to functional constraints, intrinsically limiting the flexibility of the involved pathways, and therefore potentially constraining behavioural flexibility. Over development, change in one of the multiple components of the closely coordinated neuroendocrine system could necessitate change in other components, which would imply substantial cumulative costs (Duckworth, 2015). Altogether, the physical bases of behaviour could produce a developmental bias towards behavioural consistency by limiting flexibility in developmental pathways, even without selection for behavioural consistency per se.

Accordingly, despite change being an essential element of development, stability of behaviours and personality traits between life stages have been found in a variety of species of taxa (Table 1). What is perhaps surprising is that consistency in personality across life stages has been found even in animals that have complex life cycles, for example across metamorphosis in anurans (lake frog, *Rana ridibunda*: Wilson & Krause, 2012); and in hemimetabolous insects, i.e.

those with three distinct developmental stages: egg, larva (also known as nymph) and adult (damselfly, *Lestes congener*: Brodin, 2009; field cricket, *Gryllus integer*: Hedrick & Kortet, 2012; Niemelä et al., 2012; firebug, *Pyrrhocoris apterus*: Gyuris et al., 2012). It is possible that this stability is due to some traits having a genetic (pleiotropic) or mechanistic (hormonal, neuronal, metabolic) basis that is not easily uncoupled across development (Duckworth, 2010; Sih et al., 2004).

In other species, though, behavioural consistency between life stages has not been found. Relatively few studies have reported no consistency at all, although some have found consistency in only some of the traits measured. It is possible that the majority of studies have found consistency in part due to publication bias in favour of positive results; and yet there seems to be a pattern in the species where lack of consistency is reported. In holometabolous insects –those undergoing complete metamorphosis (i.e. with four stages: egg, larva, pupa and adult)– only 4 studies have examined behavioural consistency across life stages, and three of them have found that, while larvae and/or adults showed repeatable inter-individual differences in behaviour, there is a lack of correlation between personalities across different life stages (leaf beetle, *Phaedon cochleariae*: Müller & Müller 2015; red flour beetle, *Tribolium castaneum*: Wexler et al. 2016; mealworm beetle, *Tenebrio molitor*: Monceau et al. 2017; but see ladybird beetle, *Eriopis connexa*: Rodrigues et al. 2016). The lack of consistency in behaviour across life stages is particularly interesting in the case of *Tenebrio molitor*, since larvae and adults live in the same environment with overlapping generations and low dispersal, exploit the same resources, and are thus exposed to similar environmental constraints (Monceau et al., 2017). This allows us to disentangle the effects of rapid changes in morphology and physiology over metamorphosis from the different ecological conditions experienced by different life stages in most species undergoing metamorphosis. The lack of consistency in this species suggests that the functional reorganization

occurring during metamorphosis is behind the long-term inconsistency of these traits in this—and potentially other—species (Mather & Logue, 2013), despite similar environmental pressures in different life stages. Indeed, the complete metamorphosis of holometabolous insects includes a profound remodelling of the nervous system (Consoulas et al., 2000; Tissot & Stocker, 2000), although to what extent this affects personality is unclear. At least some degree of stability should still be possible, since previous experiments in *Tenebrio molitor* have shown that learning that occurs in the larval stage persists into adulthood (Borsellino et al., 1970).

Table 1

A selection of studies examining consistency of personality across development

Species	Repeatability found across life stages	References
Mollusc		
Dumpling squid (<i>Euprymna tasmanica</i>)	+	Sinn et al. 2008
Arthropod		
Cockroach (<i>Diploptera punctata</i>)	+	Stanley et al., 2017
Damselfly (<i>Lestes congener</i>)	+	Brodin, 2009
Field cricket (<i>Gryllus integer</i>)	+ + (females) - (males)	Niemelä et al., 2012 Hedrick & Kortet, 2012
Firebug (<i>Pyrrhocoris apterus</i>)	+	Gyuris et al., 2012
Leaf beetle (<i>Phaedon cochleariae</i>)	-	Müller & Müller, 2015
Ladybird beetle (<i>Eriopsis connexa</i>)	+	Rodrigues et al., 2016
Red flour beetle (<i>Tribolium castaneum</i>)	-	Wexler et al., 2016
Mealworm beetle (<i>Tenebrio molitor</i>)	-	Monceau et al., 2017
Fish		
Sheepshead swordtail (<i>Xiphophorus birchmanni</i>)	+	Boulton et al., 2014
Brown trout (<i>Salmo trutta</i>)	+	Adriaenssens & Johnsson, 2013
Eastern mosquitofish (<i>Gambusia holbrooki</i>)	+	Polverino et al., 2016
Midas cichlid (<i>Amphilophus citrinellus</i>)	+	Francis, 1990
Amphibian		
Lake frog (<i>Rana ridibunda</i>)	+	Wilson & Krause, 2012

Table 1 (continued)

Reptile		
Common lizard (<i>Lacerta vivipara</i>)	+	Cote & Clobert, 2007
Viviparous lizards (<i>Zootoca vivipara</i>)	+	Le Galliard et al., 2013
Garter snake (<i>Thamnophis ordinoides</i>)	+	Brodie, 1993
Mexican black-bellied garter snake (<i>Thamnophis melanogaster</i>)	+	Herzog & Burghardt, 1988
Bird		
Red jungle fowl (<i>Gallus gallus</i>)	+ (females) - (males)	Favati et al., 2015
Zebra finch (<i>Taeniopygia guttata</i>)	+	David et al., 2012; McCowan & Griffith, 2014; Wuerz & Krüger, 2015
Common raven (<i>Corvus corax</i>)	+	Miller et al., 2016
Hooded crow (<i>Corvus corone corone</i>)	+	Miller et al., 2016
Mammal		
Rat (<i>Rattus norvegicus domesticus</i>)	+ -	Ray & Hansen, 2003 Rödel & Meyer, 2011
Eurasian harvest mouse (<i>Micromys minutus</i>)	+	Schuster et al., 2017
Common vole (<i>Microtus arvalis</i>)	+	Herde & Eccard, 2013
European rabbit (<i>Oryctolagus cuniculus</i>)	+	Eccard & Rödel, 2011
Yellow-bellied marmot (<i>Marmota flaviventri</i>)	+	Petelle et al., 2013
Cattle (<i>Bos taurus</i>)	+	van Reenen et al., 2013
Pig (<i>Sus scrofa domesticus</i>)	+	Janczak et al., 2003
Roe deer (<i>Capreolus capreolus</i>)	+	Debeffe et al., 2015
Horse (<i>Equus ferus caballus</i>)	+	Lansade & Bouissou, 2008; Lansade et al., 2008b, 2008a; Visser et al., 2001
Domestic cat (<i>Felis silvestris catus</i>)	+	Lowe & Bradshaw, 2001, 2002
Rhesus macaques (<i>Macaca mulatta</i>)	+	von Borell et al., 2016
Chimpanzee (<i>Pan troglodytes</i>)	+	Webb et al., 2017

Table 1. Examples of studies examining consistency of personality across development in a variety of taxa. A ‘+’ indicates that consistency was found in at least one behaviour or personality trait across different life stages, a ‘-’ indicates that no such consistency was found. Note, however, that in some studies where consistency was found across development, other behaviours were also measured that were found *not* to have consistency.

Some methodological considerations for the study of personality across development

The study of behavioral consistency across development can be challenging since it must deal head-on with the essential property of change: throughout the lifetime, individuals can face radically different internal (perceptual, physiological) and external (ecological, social) conditions. Longitudinal research is also necessary to examine stability of behaviour throughout

the lifespan, since cross-sectional studies (while valuable for examining personality at each life stage) exclude within-individual variation between developmental stages (Trillmich et al., 2015). Therefore, any behavioral traits used to examine personality throughout development must be carefully considered, as these behaviours must be relevant to the species at each stage of life under study. Moreover, the inclusion of very early life stages can present additional difficulties, for example the sometimes limited behavioural repertoire of young animals. Some behaviours are not observed in early life stages, either because the animals lack the necessary motor coordination to carry them out, they learn the behaviours only later in life, or they don't perform them because the early environment lacks the stimuli necessary to elicit them (e.g. Smotherman & Robinson, 1989). Behaviour is particularly limited in altricial species, which undergo more extensive postnatal neuromuscular development than precocial species (Muir, 2000). In these cases, the inclusion of early life stages in a study can be done using simple behavioural measures, such as separation/distress calls (e.g. Hudson et al., 2017) or activity which requires little motor control (e.g. McCowan & Griffith, 2014).

Personality can be thought of as a statistical phenomenon, in the sense that examining the consistency of an individual's behaviour only makes sense in comparison with other individuals. The most common statistic used in personality studies is repeatability (the intra-class correlation coefficient, ICC), a population-level estimate which quantifies the proportion of the total observed behavioural variation that is attributable to between-subject variation. This is usually done using variance components extracted from analysis of variance (ANOVA), or from linear or generalized linear mixed-effects model (LMM and GLMM) based methods. The estimation of repeatability therefore requires repeated measurements of multiple individuals' behavioural responses. And yet, a recent review found that only 62% of empirical personality studies used repeated measures (52% measured traits only once and 10% based their statistical analysis on a single measurement, e.g.

the average, per trait, despite having repeated measurements; Niemelä & Dingemanse 2018). Sometimes a given behaviour is presumed to be repeatable because it was found to be so in previous studies, but often the assumption is made without justification. Studying the long-term stability of personality using one measurement per life stage also assumes that inter-individual variation is stable within life stages. However, in a meta-analysis, Bell et al. (2009) found suggestive evidence that repeatability might be lower in juveniles versus adults, perhaps due to the rapid and dramatic developmental change that juveniles go through. If there is no behavioural stability within a given life stage, but it is assumed that there is and only one measurement is used, this will greatly limit the interpretation of the study and can lead to erroneous conclusions. The use of single measurements is also potentially problematic if the existence of behavioural syndromes is to be tested, since the relationship between variables at the population level can be independent (even opposite) of their relationship at the individual level (Niemelä & Dingemanse, 2018; van de Pol & Wright, 2009). For example, we might observe a population-level positive correlation between aggression and exploration, but repeated measures could show that at the individual level there is no association between the two, or there is even a negative correlation. However, most of the research on behavioural syndromes uses population-level correlations between behavioral traits as a proxy for the between-individual correlation across traits, and many researchers do not make clear at what level they interpret these correlations (Niemelä & Dingemanse, 2018).

Another consideration to be made when designing a study of personality is the method used to gather data for a study. One option is to observe organisms in their normal environment (be it in captivity or free-ranging) and gather data on naturally occurring behaviours. This method is mainly used with primates (von Borell et al., 2019), typically for assessing social behaviour, although it has also been used in other species (e.g. to study exploration in the European rabbit,

Eccard & Rödel, 2011). This method has some obvious drawbacks: it is time- and labour-intensive, the environmental stimuli necessary to elicit the desired behaviours may vary stochastically, it is particularly difficult to observe the same individuals for long periods of time for longitudinal studies and, depending on the species, it may be difficult to observe the young offspring if they are in a nest or den and guarded by a parent. The main advantage, however, is that the behaviours measured using this method are presumed to have high ecological validity. The more common approach to gathering data on personality is to use experimental assays where each individual can be placed repeatedly in a standardized test environment and be exposed to the chosen stimuli in a controlled manner, thereby evoking the desired behaviour. Whether using wild or captive animals, behavioural assays usually involve removing the animals from their normal environment and placing them in a test arena or apparatus. Sometimes, although not always, they are acclimated to the apparatus for a few minutes before their behaviour is measured, e.g. in a novel object or predator simulation test. Even with short acclimation periods, the novelty of the testing arena or apparatus, the social isolation (which is particularly important in the case of young animals which are dependent on parent(s) and may spend all their time with siblings) and the disturbance of being handled will always be potential confounding factors, difficulting the interpretation the underlying motivation of behaviour. Care should be put into controlling for these elements whenever possible through experimental design, for example by testing in the animal's home environment rather than a novel environment, which can produce different results (e.g. Beckmann & Biro, 2013). In the field, assays may be used that do not require capturing the animals and placing them in novel environments, e.g. by measuring flight initiation distance (Biro, 2013).

Once behaviours have been measured and found to be repeatable, there still remains the difficulty of ascribing the proper meaning to them. Does activity in an open field reflect exploration (e.g. Dingemanse, 2002; Rödel & Meyer, 2011), or boldness (e.g. Forsatkar et al., 2016)? Could

the individuals who move the most in the open field simply be more active (e.g. Boon et al., 2008), or are they reacting fearfully (e.g. Buijs & Tuytens, 2015)? The main advantage to using standardized tests such as the open field and the novel object is to facilitate cross-species comparison. However, since the underlying motivation of behaviour in a particular test varies depending on the species used, it could be more advantageous to design a test which is species-specific and more easily interpretable and do away with the “standard” tests altogether. An interesting area of research is to validate typical behavioural assays against behaviour observed in the field to examine their ecological validity, and against physiological measures to validate their emotional/ motivational interpretation. For example, chacma baboons (*Papio ursinus*) exposed to a novel object test and a threatening stimulus (a taxidermized venomous snake)—both assays which are typically used to measuring boldness— showed uncorrelated responses across tests, suggesting that in fact the two tests actually measure different aspects of personality (Carter et al., 2012). At another level, behavioural measures during assays can be compared with physiological measures indicative of stress, such hypothalamic-pituitary-adrenal (HPA) axis response or hypothalamic-pituitary-gonadal (HPG) axis activity, which are correlated to personality in a variety of taxa (Caramaschi et al., 2013). This is useful for validating the interpretation of behaviour, by examining the underlying emotionality of animals. Rather than difficulting comparisons between species, the use of ecologically relevant assays and behaviours, particularly in combination with validation using physiological parameters, will allow us to draw wider and more robust conclusions about personality and its significance.

Conclusions

Compared to the wealth of animal personality studies conducted on adult animals, there are still relatively few that focus on development. The change in niches, environments, and some

physiological, endocrine, neural and morphological aspects across development would seem to point to instability of personality over the lifetime; and yet the early development and constrained speed and extent at which of the underlying physical components can change (e.g. neural and endocrine tissue) points towards stability. Conflicting results in different species, coupled with the difficulties inherent in longitudinal studies and cross-species behavioural comparisons, have left us with a poor understanding of how personality changes over ontogeny. Alongside the examination of life-history and fitness traits, environmental effects, and physiological and neurobiological correlated, a developmental approach provides an opportunity for elucidating the mechanisms and evolution of personality.

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Capítulo II

Personalidad en crías del gato doméstico

Emergence of personality in weaning-age kittens of the domestic cat?

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RESEARCH ARTICLE

Emergence of personality in weaning-age kittens of the domestic cat?

Andrea Urrutia^{1,2}  | Oxána Bánszegi²  | Péter Szenczi³  | Robyn Hudson² 

¹Posgrado en Ciencias Biológicas, Unidad de Posgrado, Edificio A, 1er Piso, Circuito de Posgrados, Ciudad Universitaria, Mexico City, Mexico

²Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico

³CONACYT - Instituto Nacional de Psiquiatría Ramón de la Fuente Muñiz, Unidad Psicopatología y Desarrollo, Mexico City, Mexico

Correspondence

Andrea Urrutia, Posgrado en Ciencias Biológicas, Unidad de Posgrado, Edificio A, 1er Piso, Circuito de Posgrados, Ciudad Universitaria, Coyoacán, CP 04510, Mexico City, Mexico.

Email: andreaurrutia@outlook.com

Robyn Hudson, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, AP 70228, CP 04510, Mexico City, Mexico.

Email: rhudson@biomedicas.unam.mx

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Abstract

Individual differences in behavior (animal personality) have recently received much attention although less so in young mammals. We tested 74 preweaning-age kittens from 16 litters of domestic cats in five everyday contexts repeated three times each across a 3-week period: a handling test where an experimenter held the kitten, a test where a piece of raw beef was given to the kitten and gradually withdrawn, a test where the kitten was presented with a live mouse in a jar, a test where the kitten was briefly confined in a pet carrier, and an encounter with an unfamiliar human who first remained passive and then attempted to stroke the kitten. We found consistent individual differences in behavior in all tests except with the mouse, although less marked than in equivalent tests with adult cats. Differences in behavior were unrelated to sex, body mass, litter size, or maternal identity. We found only weak correlations in results among the tests (behavioral syndromes), again unlike findings in adult cats. We conclude that weanling kittens show consistent individual differences in behavior but in a different manner to adults. If and how the pattern of such differences changes across development remains to be studied.

KEYWORDS

behavioral syndromes, domestic cat, early development, *Felis silvestris catus*, individual differences, personality, weaning

1 | INTRODUCTION

In the last decades, the field of animal personality has attracted considerable attention from behavioral biologists, and a large number of studies have been published on the consistency of individual differences in behavior over time and across contexts. Related to animal personality are behavioral syndromes; suites of correlated behaviors across two or more trials, either in the same or different contexts or situations (Sih et al., 2004).

Much of the work on animal personality has been conducted on adult animals, with young age classes somewhat underrepresented in

the literature (Groothuis & Trillmich, 2011; Stamps & Groothuis, 2010; Trillmich & Hudson, 2011). However, the study of personality in early life stages is of particular interest since mortality rates in early life are often high and a large proportion of animals do not reach maturity (Sibly et al., 1997), resulting in intense selective pressure during development. Thus, if differences in personality are adaptive, we can expect individual differences in behavior among conspecifics to appear early in life, and indeed there is evidence of personality in young animals in a range of taxa (birds, e.g., McCowan & Griffith, 2014; mammals, e.g., Neave et al., 2020; Pérez Manrique et al., 2019, 2021; Schuster et al., 2017). Additionally, behavioral syndromes have been documented in

young animals of several species (e.g., fairy-wrens *Malurus cyaneus*, Hall et al., 2015; red squirrels *Tamiasciurus hudsonicus*, Kelley et al., 2015). However, the presence of consistent individual differences in behavior and behavioral syndromes at one life stage does not necessarily imply that such characteristics will be consistent across the lifetime. Hence, studies during development and across different age classes are necessary for a fuller understanding of the emergence and meaning of such differences (Cabrera et al., 2021).

The domestic cat *Felis silvestris catus* is an obligate carnivore (Bradshaw et al., 1996) and is generally considered only a facultatively social animal (Turner, 2017). The cat's rich behavioral repertoire and natural demanding lifestyle makes it a good model for the study of animal personality, attested by several reports using a variety of approaches (observation, owner surveys, behavioral testing) although mainly in adult animals (reviewed in Gartner, 2015; Gartner & Weiss, 2013; Mendl & Harcourt, 2000).

To our knowledge, there have been few studies of personality in kittens which test them repeatedly, and none in which they are tested repeatedly in more than one context. Nevertheless, very young kittens (less than one month of age) already show consistent individual differences in the amount of general motor activity in the nest environment (Raihani et al., 2014), and in locomotion and the number of distress calls emitted during repeated periods of brief separation from their mother and littermates (Hudson et al., 2015, 2017). In the longer term, rank-order of friendliness toward humans is reportedly consistent between the ages of 3 and 8 months (Turner et al., 1986), and cats handled more as kittens consistently reacted more "boldly" toward humans at 4 months and 1 year, although this effect was lost by 2 years of age (Lowe & Bradshaw, 2001), although the short-term stability of these traits was not evaluated within age groups. In addition, we have previously found consistent individual differences in behavior among adult male and female mixed-breed cats of diverse origin and age repeatedly tested in a range of situations (Martínez-Byer et al., 2020; Urrutia et al., 2019).

To date, we are not aware of any work that has examined the consistency of individual differences in behavior among kittens in more than one test situation (necessary to establish the possible existence of behavioral syndromes), and in general, studies of the development of individual differences in behavior in the cat are still quite limited. Thus, the aim of this study was to investigate if consistent individual differences in behavior exist in preweaning kittens of the domestic cat in a variety of situations, using a series of standardized behavioral tests. Additionally, we compared individual differences in behavior across test situations to explore the possible existence of behavioral syndromes. To our knowledge, this is the first study to explore this in cats at such an early age.

2 | METHODS

2.1 | Study site and animals

From April 2016 to October 2017, we tested 74 mixed-breed kittens (36 males, 38 females) from 16 litters (mean litter size 4.7 ± 1.4 SD, 52

range 2–7) from six mothers (mean number of kittens per mother 12.5 ± 5.4 SD) maintained as part of a free-ranging breeding colony at a private house in Mexico City. The colony females were free to leave and enter the house at will and to mate with roaming local males. Thus, paternity of the kittens was unknown (multiple paternity is common in domestic cats; Say et al., 1999). All mothers gave birth voluntarily in the house. Animals were fed daily with canned commercial cat food and fresh meat and had permanent access to water, dry commercial cat food and toilet trays, and received regular treatment against parasites. Each litter was kept in a separate room (approximately 3 m × 3 m) in the house from birth to the end of the second postnatal month. The rooms contained a commercial foam pet bed inside a large open-top cardboard box (60 × 80 × 70 cm) with a small floor-level opening cut for the mother. The box was removed when the kittens were 4 weeks old and began leaving the nest bed, at which time various enrichment objects, a scratching board, and a toilet tray were provided. From this time, kittens were also provided daily with commercial canned cat food and had permanent access to dry commercial cat food and water. The doors to the rooms had been remodeled to 1.2 m in height so that the mothers could jump freely in and out but the kittens were unable to leave. Mothers showed little interest in the litters of other females, and communal nursing did not occur. Kittens were weighed at birth and daily thereafter to the nearest gram using digital scales to check for adequate weight gain and to habituate them to human handling. Their sex was registered, and each was fitted with a different color neck ribbon for individual identification.

Animals were under regular supervision of a qualified veterinarian. All kittens survived to weaning at 8 weeks of age when they were given away as pets with the help of local veterinarians. They were kept and treated according to the guidelines for the care and use of animals in research as published in *Animal Behaviour* (ABS, 2016), according to the relevant legislation for Mexico (National Guide for the Production, Care and Use of Laboratory Animals, Norma Oficial Mexicana NOM-062-200-1999), and approved by the Institutional Committee for the Care and Use of Laboratory Animals (CICUAL, permission ID 6315) of the Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México.

2.2 | Test procedure and behavioral measures

On postnatal weeks 5, 6 and 7, and in the absence of their mother and littermates, each kitten was given five behavioral tests intended to resemble real-life situations with which it was likely to be confronted in later life. To avoid tiring the kittens, they were given only one test each weekday so that over the 3 weeks each kitten was given each test three times 1 week apart. The sequence of tests was kept constant in order to maintain the time between repetitions at one week. Littermates were tested in random order, determined by lottery cards marked with the kittens' identities, between 11:00 h and 18:00 h by an experimenter with whom they were familiar. The trials were video recorded (Sony HDR-CX130) for later analysis (see Table 1 for behavioral measures). The five tests were as follows:

TABLE 1 Behavioral variables analyzed in each test

Behavior measured	Definition
Struggle/handling test	
Struggle (latency, s)	Lifting one of the hind paws and touching or kicking the experimenter's forearm
Meat test	
Stomp or swipe (binary)	If the kitten stomped on the floor or tongs with a forepaw and/or swiped at the tongs at least once
Carry away (binary)	If the kitten walked or ran away (>3 steps) from the experimenter, carrying the meat at any point during the test
Mouse test	
Near the mouse (duration, s)	When at least the kitten's forepaws were within one body length of the jar
Sniffing the jar (duration, s)	Sniffing the jar
Circling the jar (duration, s)	Walking around the jar while looking at or physically contacting it
Separation/confinement test	
Meows (number)	Meow-type vocalizations (Nicastro, 2004)
Motor activity (duration, s)	Displacement of any of the limbs on the floor or sides of the carrier for at least 1 s
Human approach test	
Approach score (1–5)	Maximum degree of proximity to the unfamiliar human in phase 1 of the test (see text for details)
Finger-nose contact (binary)	If the kitten established contact by touching its nose to the human's outstretched finger in phase 2 of the test
Stroke success (binary)	Whether or not the unfamiliar person was able to stroke the kitten on all nine stroking attempts in phase 3 of the test

Struggle/handling test: In domestic species, struggle/restraint tests are useful as indicators of ease of handling (Grandin, 1997). This test has been used to study individual differences in behavior in adult cats (Lowe & Bradshaw, 2002; Martínez-Byer et al., 2020) and in several other species also (e.g., piglets: Horback & Parsons, 2018; weanling rabbits: Rödel et al., 2017). A high latency to struggle is thought to represent a calm temperament (low reactivity) and greater tolerance of human handling.

Kittens were not handled for at least 2 h before testing, which took place in the kittens' room in the presence of (but facing away from) littermates, as removing the kitten to another room would have necessitated handling it before the test. The experimenter approached the kitten, stroked its back three times from the neck to the base of the tail, picked it up holding it securely with both hands around the thorax under its forelimbs, and held it at shoulder height and facing away from the experimenter (Figure 1a-1) and toward the video camera, at a distance of ~1 m. The test ended when the kitten placed a hind paw on the experimenter's arm ("struggled," Figure 1a-2). The latency to struggle was recorded for further analysis (Table 1).

Meat test: Aggressive interactions between adult cats have been observed to be related to (and to possibly maintain) feeding orders or hierarchies (Bonanni et al., 2007; Knowles et al., 2004), and kittens have been observed to defend pieces of raw beef from siblings and experimenters although they do not appear to form feeding hierarchies (González et al., 2018).

Before the test, the litter was transported to a separate room, and each kitten was brought back to its home room for individual testing. Immediately before the test, the kitten was given a small piece of raw beef (2–3 g) to eat to ensure that it was motivated to perform in the test. If it did not eat this first piece of meat, the kitten was taken back to its siblings, and the test was re-attempted after a short pause.

When the kitten had eaten the first piece of meat, the experimenter gave it a second larger piece (8–10 g). The experimenter held a pair of wooden tongs (27 cm in length) on the floor, 30 cm from the kitten's head, with the kitten facing the tongs. The test began once the kitten had started to chew on this second piece of meat. During three seconds, the experimenter slowly moved the tongs toward the kitten's face (Figure 1b-1) and used the tongs to gently grab and withdraw the meat, ending the test once the tongs had been returned to their starting point (Figure 1b-2; Table 1). If the kitten moved away carrying the meat at any point during the test (either before or after the tongs reached the kitten), this behavior was coded and the test ended (see Table 1). During the test, the experimenter knelt in front of the kitten at a distance of at least 50 cm, and the video camera was placed at ground-level perpendicular to the experimenter and kitten, at a distance of ~1 m. The kittens' reaction to the removal (or attempted removal) of the meat was scored (see Table 1 for details). Failure to attempt to retain or defend possession of the meat in response to the attempt by the experimenter to remove it is thought to represent a calm temperament (low reactivity) and greater tolerance of human presence.

Mouse test: Even naïve cats respond with marked signs of attention and approach when presented with a live mouse (reviewed in Chacha et al., 2020; Martínez-Byer et al., 2020), one of the main natural prey species of the cat (Biró et al., 2005). As obligate carnivores, cats show interest in small rodents from an early age: in laboratory conditions kittens approach live mice as early as three weeks of age (Adamec et al., 1980) and kittens have been reported to hunt and kill small mice even before two months of age (Baerends-van Roon & Baerends, 1979).

The litter was removed to a separate room, and each kitten was carried back to its home room individually to be tested. There it was placed in front of a heavy transparent glass jar (10 cm diameter x 18 cm tall)

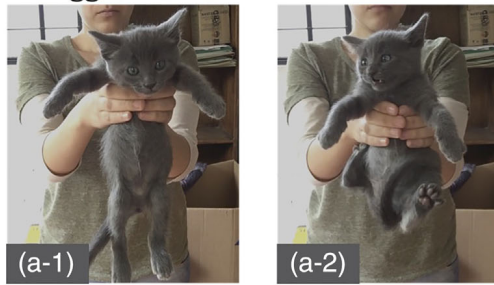
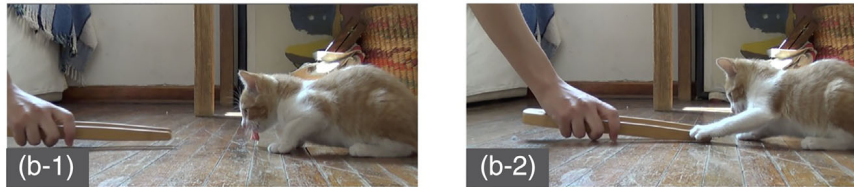
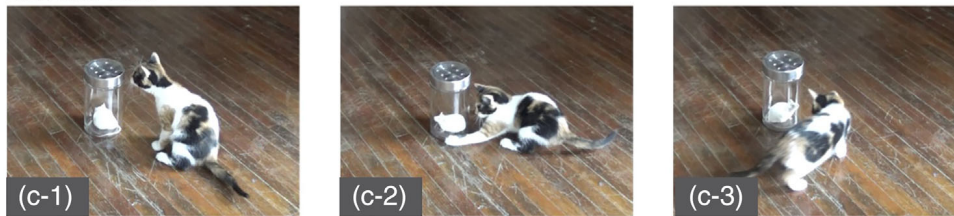
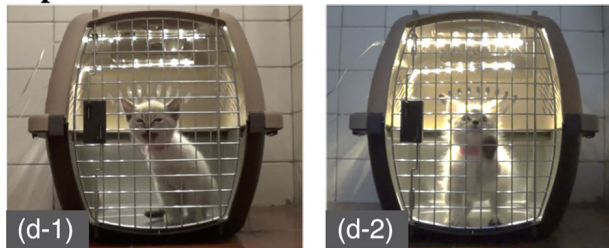
Struggle test**Meat test****Mouse test****Separation / confinement test****Human approach test**

FIGURE 1 Behavior of kittens in the five tests. (a) Struggle test. a-1. Kitten being held up by experimenter at the start of the test. a-2. Kitten struggling. (b) Meat test. b-1. Experimenter moving tongs toward kitten as it chews on a piece of beef. b-2. Kitten stomping on the tongs as the experimenter removes the meat. (c) Mouse test. c-1. Kitten sniffing the mouse jar. c-2. Kitten pawing at the mouse jar (note that pawing behavior occurred infrequently and was therefore excluded from the analysis). c-3. Kitten circling the mouse jar. (d) Separation/confinement test. d-1. Kitten meowing in the carrier. d-2. Motor activity in the carrier. (e) Human approach test. Arrows indicate the kitten. e-1. Kitten near the human. e-2. Kitten on top of the human. e-3. Finger-nose contact. e-4. Kitten being stroked

taped to the floor, containing a live white female (BALB/c) laboratory mouse (for details of housing and measures taken to minimize stress to the mice, see Martínez-Byer et al., 2020). The jar had a perforated lid allowing the kitten to smell and hear the mouse but without coming into direct physical contact with it. The kitten was placed on the

floor, ~20 cm from the jar, and left alone for 2 min (Figure 1c-1–c-3). The test was filmed with a video camera placed on a tripod ~2 m away from the mouse jar, and the kitten's reaction to the mouse was analyzed (see Table 1 for details). This test is thought to represent the degree of curiosity or boldness of the individual.

Separation/confinement test: Brief separation from conspecifics has been used to study individual differences in behavior in various mammals such as horses (Lansade et al., 2008; Pérez Manrique et al., 2019; 2021), goats (Finkemeier et al., 2019), and also in domestic kittens (Hudson et al., 2015, 2017) and adult cats (Martínez-Byer et al., 2020; Urrutia et al., 2019). Separation from familiar conspecifics and confinement is stressful and elicits distress calls in cats of all ages, and in adults has been observed to elicit motor activity such as scratching, circling, and pacing. Thus, a high number of vocalizations and high motor activity are thought to represent fearfulness or timidity and high reactivity to change in the (social) environment.

Kittens were tested in an unfamiliar room on a different level of the house to minimize auditory and olfactory contact with littermates. While the rest of the litter remained in its home room the experimenter carried the focal kitten to the test room where she locked it in a commercial pet carrier (38 × 58 × 30 cm) on the floor with a camera placed 50 cm away and facing the door of the carrier. The interior of the carrier was fitted with two LED lights to improve visibility for later video analysis. The experimenter then left the room at which point the test began. The kitten could look out of the carrier through the metal grill door and a series of small holes on the walls (Figure 1d-1 and d-2). After the kitten had remained alone in the room for 2 min, the test ended and the experimenter brought the kitten back to its home room. The interior of the carrier was wiped down with isopropyl alcohol between tests. Kittens' vocal and motor activity were analyzed (see Table 1 for details).

Human approach test: Reaction to the presence of an unfamiliar human is one of the most commonly used behavioral tests in several domestic species, for example, in horses (Lansade & Bouissou, 2008), dairy cows (Gibbons et al., 2009), and also in cats (e.g., McCune, 1995; Podberscek et al., 1991). Readiness to approach a stranger is thought to indicate curiosity and boldness in approaching and investigating a novel object and tolerance of or a positive attitude toward humans.

Before testing, the litter was taken to a separate room, and each kitten was carried back to its home room by the experimenter to be tested individually. An unfamiliar man sat cross-legged on the floor in the middle of the kittens' home room (Figure 1e-1). The experimenter entered the room with the kitten, placed it on the floor facing toward the man at a distance of 1 m, and left the room, at which point the test began. The test was filmed with a video camera placed on a tripod in a corner of the room, and facing toward the man.

Following the method used by Martínez-Byer et al. (2020), the test was divided into three phases. During the first phase, lasting 2 min, the man sat motionless looking at the wall, ignoring the kitten regardless of whether or not it approached or made contact with him (Figure 1e-1 and e-2). The experimenter then knocked lightly on the door to signal the start of the second phase, lasting 1 min, during which the person looked at the kitten, held out a hand with his index finger extended toward the kitten, and called softly "Here, kitty," "Ps-ps-ps" (Figure 1e-3). The experimenter then again knocked on the door to signal the start of the third phase. The person quietly stood up, approached the kitten and attempted to stroke it gently three times

from head to tail (Figure 1e-4). He then took a step back, waited a few seconds, and repeated this procedure twice for that trial, giving a total of nine stroking attempts. The trial ended after the last stroking attempt. The man called to the experimenter, who entered the room and returned the kitten to its littermates.

For the first phase, the kitten was assigned an "approach score." Each kitten in each trial was given the highest score it achieved from 1 to 5 as follows: (1) was 1 m or more away from the person, (2) approached to less than 1 m, (3) approached within one kitten body length (Figure 1e-1), (4) made physical contact with any part of the person (touch with paw or nose, lick, bite), (5) climbed onto the person or placed two or more paws simultaneously on him (Figure 1e-2). During the second phase, kittens received a binary score depending on whether they touched the person's finger with their nose ("finger-nose contact," Figure 1e-3) or not. During the third phase, kittens received a binary score according to whether the person was able to stroke the kitten in all nine stroking attempts of the three sessions, or not (Figure 1e-4; Table 1).

Behavioral variables were coded from the videos using Solomon Coder software (Péter, 2011). To assess inter-rater reliability, additional observers blind to the purpose of the study independently scored the kittens' behavior for a subset of videos (ranging from 19 to 87 videos, 8.5%–39% per test). Inter-rater reliability was assessed using Cohen's kappa (κ) for categorical variables and a one-way intra-class coefficient (ICC) for continuous variables using the R package *irr* (Gamer et al., 2013). One variable had only moderate inter-observer agreement (hissing/growling in the meat test, $\kappa = 0.462$, $p < .005$), probably due to the difficulty in hearing these low-volume vocalizations; hence, it was excluded from further analysis. All other variables had high inter-rater agreement ratings ranging for continuous variables from ICC = 0.876, $p < .001$ (time spent sniffing the jar in the mouse test) to ICC = 0.966, $p < .001$ (number of meows in the separation test), and for categorical variables from $\kappa = 0.762$, $p < .001$ (approach score in the human test) to $\kappa = 0.875$, $p < .001$ (finger-nose contact in the human approach test).

2.3 | Statistical analysis

This was carried out using the program R, version 3.5.2 (R Core Team, 2020).

Fixed effects: We tested for significant maternal, litter, and individual effects for each of the measured behaviors by fitting linear mixed models (LMMs) for normal and log-transformed normalized data, or generalized linear mixed models (GLMMs) for Poisson-distributed count and binary data using the *lme4* package (Bates et al., 2015) in R. Plots of residuals versus fitted values, and Q-Q plots were used to visually inspect model residuals for homogeneity of variance and normal distribution. Individual and maternal identity were included as random factors. Litter size, trial number (1 to 3), sex of the kitten, and kitten body mass at the time of testing were included as fixed effects. Litter size and body mass were centered and scaled to improve model convergence and output interpretation. Models were reduced using backward

stepwise selection, starting with all candidate variables in the model, and removing the nonsignificant variable with the highest p -value in each step, until no nonsignificant variables remained.

Repeatability: Across-trial adjusted repeatability, that is, the proportion of variance explained by the individual divided by the total phenotypic variance (Sokal & Rohlf, 1995), of each behavior was calculated using intra-class correlations obtained through LMM-based calculations for normal and log-transformed normalized data and GLMM-based calculations for count (Poisson-distributed) and binary data, using the R package *rptR* (Nakagawa & Schielzeth, 2010). Kitten identity was included in all models as a random factor and any significant fixed effects identified in the previous analysis were included in these models as appropriate. p -Values were calculated by 1000 permutations, and 95% confidence intervals (95% CI) for parameter estimates were assessed using 1000 bootstrapping runs.

Behavioral syndromes: At the between-individual level (behavioral syndromes cf. Dingemanse et al., 2012; Dingemanse & Wright, 2020; Sih et al., 2004), we assessed relationships between repeatable behavioral variables using mutual information analysis (MI; Pardy & Wilson, 2011). MI quantifies the reduction in uncertainty (entropy) of the value of one variable given the knowledge of another, and it can be used to examine nonlinear/nonmonotonic relationships. All seven of the behaviors showing repeatable individual differences (see Table 3) were included in this analysis: latency to struggle (struggle/handling test), stomping/swiping and carrying the meat away (meat test), number of meows and duration of motor activity (separation/confinement test), and approach score and finger-nose contact (human approach test) (Table 1). Behavioral variables from the mouse test were not included in this analysis because none showed repeatable individual differences. To examine only between-individual level associations between behaviors, each subject's mean value for this subset of behaviors was used, thus eliminating within-individual variation (Dingemanse et al., 2012). Each possible pair of behaviors (seven behavioral variables = 21 distinct pairs) was analyzed using jackknife-bias corrected mutual information (BCMI), calculated using the *mpmi* R package (Pardy, 2019). p -Values were calculated using 1000 permutations, and the critical α -value was adjusted for false discovery rate according to Benjamini and Hochberg (1995).

3 | RESULTS

3.1 | Repeatability of behaviors

There were significant although small effects of some fixed effects on some of the behavioral variables (Table 2). Therefore, these effects were included where applicable in the repeatability analyses as fixed effects, in addition to individual identity which was a random factor. Repeatable individual differences between kittens were found in seven of the 11 behavioral measures, and four of the five tests (Table 3, Figure 2). However, the repeatability coefficient R was relatively modest (near 0.3) for most behavioral measures, except for the number of vocalizations in the separation test which had relatively high repeatability ($R = 0.599$), and carrying away the meat in the meat test which had low repeatability ($R = 0.179$) (see Table 3 for details).

ability ($R = 0.599$), and carrying away the meat in the meat test which had low repeatability ($R = 0.179$) (see Table 3 for details).

3.2 | Behavioral syndromes

None of the 21 possible pairs of behaviors had a significant BCMI value, indicating a lack of association and thus of behavioral syndromes among any of the behaviors tested.

4 | DISCUSSION

In this study, we evaluated short-term repeatability of individual differences in behavior in preweaning age domestic kittens in five different test situations. Returning to the main aim of this study, in four of the five tests, we found repeatable individual differences, which extends our previous results on individual differences in the behavior of kittens in brief isolation tests (Hudson et al., 2015, 2017). We previously found that adult cats in animal shelters showed stable individual differences in all of the tests employed in the present study, with the exception of the meat test which was not previously performed (Martínez-Byer et al., 2020; Urrutia et al., 2019). In the present study, individual differences in most of the behaviors measured were repeatable in kittens, but with considerably lower R values than those reported for adult cats. For example, the highest R value in the present study was for the number of vocalizations emitted during separation/isolation, $R = 0.599$, which is very similar to what was previously reported in kittens in the first postnatal month (Hudson, 2015, 2017), whereas in adult cats we previously found a considerably higher $R = 0.91$ to $R = 0.92$ for this behavior (Martínez-Byer et al., 2020; Urrutia et al., 2019). Furthermore, in the present study, we did not observe any repeatable individual differences in behavior of the kittens in the mouse test, although adult cats do show such differences (Martínez-Byer et al., 2020).

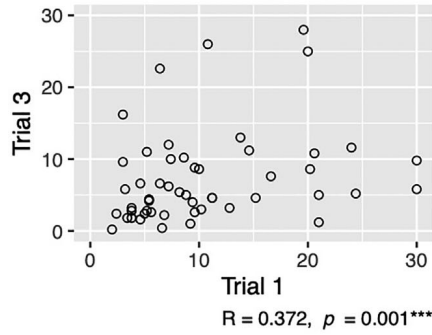
In a variety of other mammals, different aspects of individual development and the early environment have been found to affect behavior and/or personality, such as sex (e.g., Debeffe et al., 2015), birth weight and/or its deviation from the litter mean (e.g., Rödel & Meyer, 2011), litter size (e.g., Dimitsantos et al., 2007), and maternal identity (e.g., Taylor et al., 2012). However, in the present study, we found either no or negligible effects of these variables on any of the behaviors measured. Overall, the kittens in the present study exhibited repeatable individual differences in the behaviors tested independently of sex and litter effects, but in fewer behaviors and to a lesser degree than adult cats.

We suggest at least two possible explanations for finding weaker evidence of repeatable individual differences among the kittens in the present study than in our previous studies of adult cats given the same tests (Martínez-Byer et al., 2020; Urrutia et al., 2019). The first is that these preweaning kittens were still undergoing considerable motor, sensory and presumably also cognitive development across the 3 weeks of repeated testing, with various aspects of their behavior still labile and needing time to consolidate and stabilize. Given this, it might even be considered surprising that they showed consistent individual

FIGURE 2 Repeatable behaviors in the first and third trial of each test. Note that a 10% jitter was added to overlapping points for binary (meat test: carry away the meat, stomp or swipe; human approach test: finger-nose contact) and ordinal variables (human approach test: approach score) and ordinal variables (human approach test: approach score). * $p < .05$; ** $p < .01$; *** $p < .001$

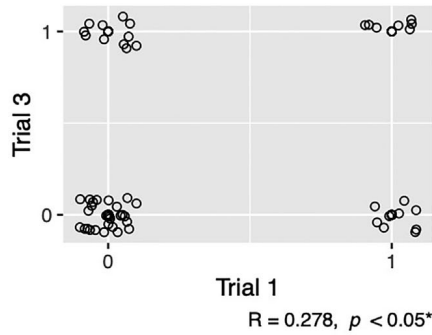
Struggle/handling test

Latency to struggle (s)

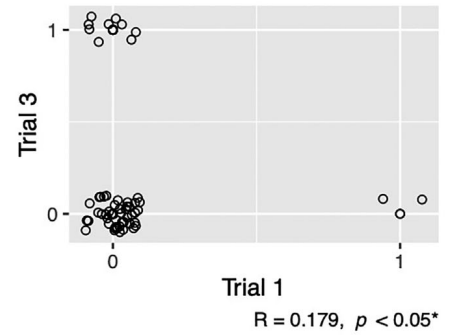


Meat test

Stomp or swipe

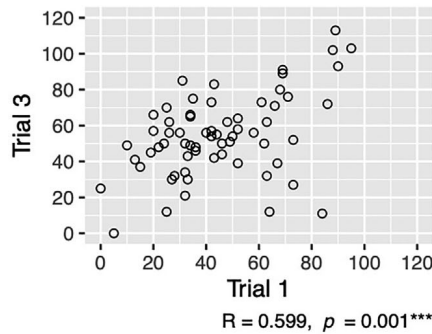


Carry away the meat

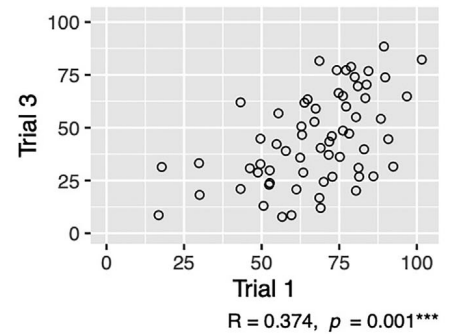


Separation/confinement test

Number of meows

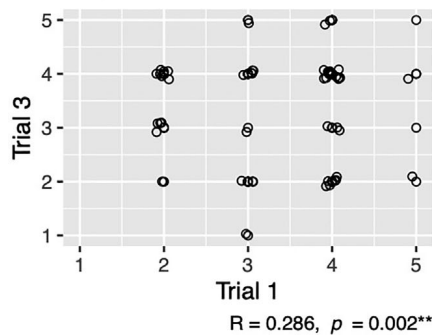


Duration of motor activity (s)



Human approach test

Approach score



Finger-nose contact

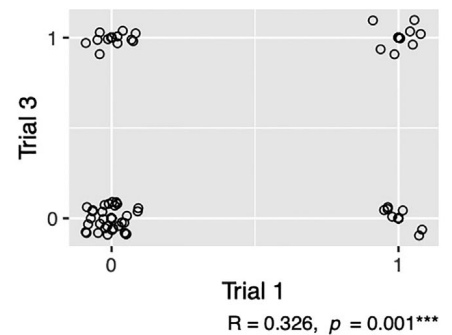


TABLE 2 Significant fixed effects resulting from fitting linear mixed models to the behavioral data. Note that some behavioral variables were log-transformed (denoted by §), and that no significant fixed effects were found for any behaviors in the meat and human approach tests

Behavior	Sum of squares	Mean squares	df	F	p
Struggle/handling test					
<i>Latency to struggle §</i>					
Trial	6.967	3.4849	2	7.958	<.001***
Mouse test					
<i>Time spent near the mouse jar</i>					
Body mass	10,193	10192.8	1	14.1	<.001***
<i>Time spent circling the jar §</i>					
Body mass	47.257	47.257	1	47.56	<.001***
Separation/confinement test					
<i>Number of meows</i>					
Trial	2677.6	1338.80	2	5.607	<.01**
<i>Duration of motor activity</i>					
Trial	14902.7	7451.3	2	31.620	<.001***
Litter size	4149.6	829.9	5	3.522	<.01**

** $p < .01$; *** $p < .001$.**TABLE 3** Repeatability of the variables analyzed for each of the behavioral tests. Repeatability (R), 95% confidence intervals (CI; lower bound, upper bound) based on 1000 bootstrap steps, and significance values (p) based on 1000 permutations are shown

Behavior	R	95% CI (lower bound, upper bound)	p
Struggle/handling test			
Latency to struggle	0.372	(0.201, .524)	.001***
Meat test			
Stomp or swipe (binary)	0.278	(0.041, .449)	.002**
Carry away (binary)	0.179	(0, 0.851)	.044*
Mouse test			
Duration near the mouse	0.123	(0, 0.268)	.05
Duration sniffing the jar	0.121	(0, 0.262)	.051
Duration circling the jar	0.184	(0, 0.345)	.380
Separation/confinement test			
Number of meows	0.599	(0.471, 0.705)	.001***
Duration of motor activity	0.374	(0.213, 0.522)	.001***
Human approach test			
Approach score	0.286	(0.126, 0.439)	.002**
Finger–nose contact (binary)	0.326	(0.076, 0.52)	.001***
Stroke success (binary)	0.13	(0, 0.153)	.396

* $p < .05$; ** $p < .01$; *** $p < .001$.

differences across the three weeks of repeated testing at all. Nevertheless, the results indicate that four of the five tests were suitable for investigating the development of individual differences in behavior in kittens from an early age, which was one of the questions of the study.

The second possible reason for the differences mentioned above between the kittens and adult cats was the kittens' relative lack of experience of a diverse world beyond the confines of their nursery room, albeit equipped with various enrichment objects. This might explain, for example, the lack of consistent individual differences on any of the measures in the mouse test. Although our free-ranging mothers certainly hunted mice, we found no indication that they brought these back to their kittens or that the kittens had any previous experience of mice at the time of testing. This was surely different from the adult shelter cats we studied previously (Martínez-Byer et al., 2020; Urrutia et al., 2019), most of which had spent at least some time as strays and, at least to some degree, had almost certainly depended on hunting mice or other small rodents for their survival (see review in Chacha et al., 2020 and for evidence of the role of experience in the choice of rodent prey in adult domestic cats).

Thus, taking the above considerations together, in future studies, we might expect more marked individual differences to emerge across development as animals gain greater experience across maturation, and possibly develop individually different behavioral strategies in coping with life's challenges (Stamps & Groothuis, 2010; Trillmich & Hudson, 2011; Wolf & Weissing, 2010).

Concerning our second aim, we did not find evidence of behavioral syndromes in the kittens. Although we found repeatable individual differences in seven of the 11 behaviors, these differences were not correlated among each other and did not organize into consistent patterns of individual differences across inter-related behaviors. This is in contrast to our previous findings in adult cats, where behavioral syndromes were identified and appeared to largely relate to human-oriented behaviors (Martínez-Byer et al., 2020). The discrepancy between the lack of behavioral syndromes in kittens compared to those found in (different) adult cats appears to be consistent with a recent cross-species

review (Cabrera et al., 2021), which found that behavioral syndromes appear to rarely be stable throughout development; more often, they emerge at certain ages or weaken over time. Related to the possible role of experience in consolidating individual differences in behavior mentioned above, the lack of syndromes might have been due to the kittens' limited experience of human contact at this early age. Apart from daily weighing, basic maintenance procedures, and testing, handling the kittens was kept to a minimum to standardize procedures and maintain semi-naturalistic developmental conditions.

To our knowledge, only two longitudinal studies of individual differences in behavior have been conducted in domestic cats. Lowe and Bradshaw (2001) recorded the postfeeding behavior of individual cats at 4 months, 1 year, and 2 years of age and found consistency between these ages (especially in the cats' behavior toward the observer). Lowe and Bradshaw (2002) also reported the results of a handling test by an unfamiliar person on a cohort of cats at 2, 4, 12, 24, and 33 months of age, and with the exception of the 12-month period, found consistency in individual differences in attempts to escape and signs of stress between ages. To better understand how individual differences in behavior develop in the cat and other species, and to extend previous work on the cat and address the discrepancy between the results of the present study and those of our previous work on adult cats (Martínez-Byer et al., 2020), more extensive longitudinal research is needed. Whether individual differences in behaviors in the domestic cat are consistent before and after major transitional and developmental periods, such as weaning and sexual maturity, and consolidate to form behavioral syndromes remains to be seen. A report of one such study is currently in preparation in which a subset of the kittens used in the present study were repeatedly tested into adulthood using the same behavioral methods as reported here, together with additional physiological measures.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

ORCID

Andrea Urrutia  <https://orcid.org/0000-0001-5936-9740>

Oxána Bánszegi  <https://orcid.org/0000-0002-1704-3539>

Péter Szenczi  <https://orcid.org/0000-0002-8495-1633>

Robyn Hudson  <https://orcid.org/0000-0002-5526-9555>

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Capítulo III

Desarrollo de la personalidad en el gato doméstico: Un estudio longitudinal

Development of “personality” in the domestic cat: A longitudinal study

Manuscrito en preparación para *Developmental Psychobiology*

Development of “personality” in the domestic cat: A longitudinal study

Andrea Urrutia^{1,2*}, Oxána Bánszegi², Péter Szenczi³, Robyn Hudson^{2*}

¹Posgrado en Ciencias Biológicas, Unidad de Posgrado, Edificio A, 1er Piso, Circuito de Posgrados, Ciudad Universitaria, Coyoacán, CP 04510, Mexico City, Mexico;
andreaurrutia@outlook.com

²Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, AP 70228, CP 04510, Mexico City, Mexico; oxana.banszegi@gmail.com,
rhudson@biomedicas.unam.mx

³CONACYT - Instituto Nacional de Psiquiatría Ramón de la Fuente Muñiz, Unidad Psicopatología y Desarrollo, Calz. México-Xochimilco 101, CP 14370, Ciudad de México, Mexico; peter.szenczi@gmail.com

Running title: Development of cat personality

*Correspondence: andreaurrutia@outlook.com, Tel: + 52 56 22 38 28;
rhudson@biomedicas.unam.mx, Tel: + 52 56 22 38 28

ABSTRACT

Although individual differences in behavior (animal personality) have recently received much attention, their development remains understudied. We have previously found, in 74 2-month-old domestic kittens from 16 litters, consistent individual differences in behavior in four tests simulating everyday contexts. In the present study, we followed a subset of these same cats and repeated the same tests at 6, 12 and 18 months of age. Behavior become increasingly repeatable with age, due to a combination of decreased individual-level variance (canalization) and increased among-individual variance; these changes in variance and repeatability continued into adulthood (12-18 months). We did not observe behavioral syndromes at any age, in contrast to previous reports in a different population of adult cats. The mechanisms that drive increased repeatability with age, and the possibility of personality structure differing between populations in this species remain to be studied.

Keywords: domestic cat, ontogeny, *Felis silvestris catus*, individual differences, repeatability, canalization, behavioral syndromes

1 INTRODUCTION

Animal personality refers to individual differences in behavior which remain consistent over time (Réale et al., 2010; Roche et al., 2016). Most of the research in this field to date has been conducted on adult animals, and an even greater proportion of it is cross-sectional, i.e. personality is evaluated at a single point in time or over very short time scales (Bell et al., 2009), therefore excluding individual variation between life stages (Trillmich et al., 2015; Cabrera et al., 2021). However, studies that span different developmental stages are necessary for a fuller understanding of animal personality and behavioral syndromes, and their proximate (e.g. genetic, neuroendocrine, environmental, developmental) and ultimate (ecological and evolutionary) causes and consequences. In an applied context, a better understanding of their development could be beneficial for domestic animal husbandry practices and selective breeding programs (Boissy et al., 2005; Conrad et al., 2011). However, it is only recently that attention has been turned towards studying personality across ontogeny (Cabrera et al., 2021).

The existence of animal personality is typically assessed by calculating the repeatability of behaviors, which is the proportion of total variance of a trait that is attributable to among-individual differences (Sokal and Rohlf, 1995); high repeatability indicates that there are stable inter-individual differences in a trait. The repeatability of a behavior is calculated by dividing its among-individual variance between its total variance (the sum of among-individual variance plus within-individual, or residual, variance):

$$R = \frac{\textit{Among-individual variance}}{\textit{Among-individual variance} + \textit{Within-individual variance}}$$

Therefore, a high repeatability value can be caused by high among-individual variance and/or low within-individual variance. It is important for developmental studies of personality to not only report repeatability but also these variance components, since they can be influenced by

independent mechanisms and thus can have different biological implications: these variance components can change across development due to processes of canalization, state-dependent feedback, and developmental plasticity (Trillmich and Hudson, 2011; Bateson et al., 2014; Sih et al., 2015; Westneat et al., 2015; Stamps and Biro, 2016; Kok et al., 2019).

Canalization was originally described by Waddington (1942) as the developmental process wherein a specific genotype follows the same trajectory even under different conditions. It can be more broadly understood as the reduction of within-individual (residual) phenotypic variation against environmental or other perturbations, through any developmental mechanism (Westneat et al., 2015). On the among-individual level, developmental plasticity can encourage divergence (a ‘fanning out’) of behavior between individuals over ontogeny, due to feedback mechanisms which act on differences that were present early in life and, over time, affect behavior (or “personality types”) later in life (Sih et al., 2015; Stamps and Biro, 2016).

In addition to the repeatability of individual behavioral traits, a central interest of animal personality research is that of behavioral syndromes: two or more behavioral traits that are correlated consistently between individuals (Sih et al., 2004). Few studies have examined the structure of behavioral syndromes across development, with mixed results. In some species, behavioral syndromes are present and persist between life stages (e.g. damselfly, *Lestes congeneris*: Brodin, 2009), in others they are absent (e.g. zebra finches, *Taeniopygia guttata*: Wuerz and Krüger, 2015), and sometimes a behavioral syndrome is present in juvenile and adult life stages, but absent during an intermediate period of ‘personality restructuring’ (e.g. Northern common boa, *Boa imperator*: Simková et al., 2017). Behavioral syndromes could be consistent across development if the traits involved share a genetic (pleiotropic) or mechanistic (hormonal, neuronal, metabolic) basis that is not easily uncoupled (Sih et al., 2004; Duckworth, 2010).

The domestic cat *Felis silvestris catus* is an obligate carnivore (Bradshaw et al., 1996) and a facultatively social animal (Turner, 2017). Its rich behavioral repertoire and relevance as a companion animal have made it an attractive model for the study of animal personality, and previous works on this subject have employed a variety of approaches (observation, owner surveys, behavioral testing) although mainly on adult animals (reviewed in: Mendl and Harcourt, 2000; Gartner and Weiss, 2013; Gartner, 2015; Travník et al., 2020). Although very few studies have examined the long-term consistency of behavioral traits in the cat, it appears that certain human-oriented behavior may be stable between kittenhood and adulthood. In one study, independent observers ranked 22 kittens according to their friendliness towards humans, and these rankings were consistent between the ages of 3 and 8 months (Turner et al., 1986). In a second study, Lowe and Bradshaw (2002) evaluated the response of cats kept as companion animals towards being briefly restrained and stroked by an experimenter at the ages of 2, 4, 12, 24 and 33 months, spanning life stages from weanling to adult. Only one trial was performed at each stage, so short-term repeatability (within life stages) cannot be inferred from this study; however, consistency in the cats' responses was observed from 4-33 months. Finally, Lowe and Bradshaw (2001) studied cats after eating a meal at the ages of 4, 12 and 24 months, scoring behaviors such as staying indoors after eating, and rubbing against people and objects. In general, they found stability of these behaviors across different age classes, although again, within-age stability was not evaluated.

In previous research by our group, we have found repeatable individual differences in behavior in pre-weaning aged domestic kittens tested in a range of ecologically relevant situations, but no behavioral syndromes (Urrutia et al., 2022). We have also previously found consistent individual differences in behavior among adult mixed-breed cats tested in the same situations, and evidence of a behavioral syndrome which appeared to be driven by differences in human-oriented

behaviors (Urrutia et al., 2019; Martínez-Byer et al., 2020). Finally, we have found both short- and long-term consistency of behavior in a single, stressful test repeated multiple times during development, at 2, 6, 12 and 18 months of age (Urrutia et al, submitted). The emergence and/or change of behavioral syndromes over time, however, has never been examined before in this species.

The aim of this study was to investigate if the consistent individual differences in behavior exhibited by pre-weaning domestic kittens in a series of standardised behavioral tests remain consistent across development into adulthood. Additionally, we aimed to explore the possibility that behavioral syndromes, which were absent during early life, may emerge during later development.

2 METHODS

2.1. Study site and animals

From April 2016 to October 2017, 74 mixed-breed kittens (36 males, 38 females) from 16 litters (mean litter size 4.7 ± 1.4 *SD*, range 2 – 7) were born from six mothers (mean number of kittens per mother 12.5 ± 5.4 *SD*) maintained as part of a free-ranging breeding colony at a private house in Mexico City. For details on how cats were kept, see Urrutia et al. (2022). On postnatal weeks five, six and seven, and in the absence of their mother and littermates, each kitten was tested three times in four behavioral tests as described below. At 8 weeks of age the kittens were given away as pets to people living in Mexico City. All cats were neutered soon after adoption except for one male and one female. However, as removing them from the analysis had no effect on the results, we retained their data to maximize sample sizes.

A subset of these cats was later visited in their new homes at the ages of 6, 12, and 18 months (see Supplementary Material Table 1 for details of sample sizes). For these cats, at each age, three visits to their homes were arranged to repeat the tests. Due to technical problems resulting in data loss, some data from some trials are missing, and additionally, not all cats in the older age groups were available for all trials. In these cases, analyses were carried out with the data available.

2.2. Test procedure

For the kittens, on postnatal weeks five, six and seven, and in the absence of their mother and littermates, each individual was given one test per day, so that over the three weeks each kitten was given each test three times one week apart. For further details on how the kittens were tested, see Urrutia et al. (2022). After weaning and adoption at 2 months of age, the same tests were carried out in the cats' new homes at the ages of 6, 12, and 18 months. Each cat was visited for testing in its home three times at each age group, leaving 1 to 6 days between visits. On a testing day, each of the four tests was performed once in an unoccupied room of the home; the cats were given a 2-3 minute break between tests for the first three tests, and a 5-10 minute break before the final test. All trials were video recorded (Sony HDR-CX130) for later analysis (see Table 1 for behavioral measures). The four tests are described below, in the order in which they were conducted. For further details on the justification for using these tests, see Urrutia et al. (2022).

Struggle/handling test. The experimenter approached the cat, stroked its back three times from the neck to the base of the tail, picked it up holding it securely with both hands around the thorax under its forelimbs, and held it at shoulder height and facing away from the experimenter and

towards the video camera, at a distance of ~1 m. The test ended when the cat placed a hind paw on the experimenter's arm. The latency to struggle was recorded (Table 1).

Meat test. The experimenter gave the cat a small piece of raw beef (8-10 g) and held a pair of wooden tongs (27 cm in length) on the floor, 30 cm from the cat's head, with the cat facing the tongs. After the cat began to chew on the meat, the experimenter slowly (over 3 s) moved the tongs towards the cat's face and used the tongs to gently grab and withdraw the meat, ending the test once the tongs had been returned to their starting point. If the cat moved away carrying the meat at any point during the test (either before or after the tongs reached the cat), this behavior was coded and the test ended (see Table 1). During the test the experimenter knelt in front of the cat at a distance of at least 50 cm and the video camera was placed at ground-level perpendicular to the experimenter and cat, at a distance of ~1 m. The cat's reaction to the removal (or attempted removal) of the meat was scored (see Table 1 for details).

Mouse test. The cat was presented with a live white female (BALB/c) laboratory mouse placed in a heavy transparent glass jar (10 cm diameter x 18 cm tall) taped to the floor (for details of housing and measures taken to minimize stress to the mice, see Martínez-Byer et al., 2020). The jar had a perforated lid allowing the cat to smell and hear the mouse without coming into direct physical contact with it. The cat was placed on the floor, ~20 cm from the jar, and left alone in the test room for two minutes. The test was filmed with a video camera placed on a tripod ~2 m away from the mouse jar, and the cat's reaction to the mouse was analysed (see Table 1 for details).

Separation/confinement test. The experimenter locked the cat in a commercial pet carrier (38 x 58 x 30 cm) on the floor of the test room with a camera placed 50 cm away and facing the door of the

carrier. The interior of the carrier was fitted with two LED lights to improve visibility for later video analysis. The experimenter then left the room at which point the test began. The cat could look out of the carrier through the metal grill door and a series of small holes on the walls. After the cat had remained alone in the room for two minutes, the test ended. The interior of the carrier was wiped down with isopropyl alcohol between tests. The cat's vocal and motor activity were analysed (see Table 1 for details).

2.3. Data processing and statistical analysis

Behavioral variables were coded from the videos using Solomon Coder software (Péter, 2011) as described in Table 1. All data analysis was performed using the program R, version 4.1.2 (R Core Team, 2021).

Fixed effects. We tested for significant effects of trial number, sex, age group (2, 6, 12 and 18 months), the interaction of age and sex, litter size and body mass at 1 week of age for each of the measured behaviors; we also tested for an effect of the cats' housing conditions (indoor or indoor-outdoor) on the behaviors measured in the mouse test. We fitted linear mixed models (LMMs) for normal and log-transformed normalized data and generalized linear mixed models (GLMMs) for Poisson-distributed count and binary data using the *lme4* package (Bates et al., 2015) in R. Plots of residuals versus fitted values and Q-Q plots were used to visually inspect model residuals for homogeneity of variance and normal distribution. Individual identity was included as a random factor. Models were reduced using backwards stepwise selection, starting with all candidate variables in the model, and removing the non-significant variable with the highest *p*-value in each step, until no non-significant variables remained.

Repeatability. Repeatability was calculated for each behavioral variable within each age group, and across age groups. This was done in two ways: first, agreement repeatability, where only the individual identity was included as a random factor, and no fixed effects were added; and second, adjusted repeatability, where individual identity was included as a random factor, and any additional fixed found to have a significant effect in the G/LMMs described above were included where applicable (Nakagawa and Schielzeth, 2010). Repeatabilities were calculated using intra-class correlations obtained through LMM-based calculations for normal and log-transformed normalized data and GLMM-based calculations for binary data, using the R package *rptR* (Nakagawa and Schielzeth, 2010). *P*-values were calculated by 1,000 permutations, and 95% confidence intervals (95% CI) for parameter estimates were assessed using 1,000 bootstrapping runs. Additionally, the among-individual and within-individual (residual) variance components of the agreement repeatability calculations were obtained (Stoffel et al., 2017). Finally, agreement repeatability was calculated between consecutive age groups: 2-6, 6-12 and 12-18 months.

Behavioral syndromes. At the among-individual level (behavioral syndromes cf. Sih et al., 2004; Dingemanse et al., 2012; Dingemanse and Wright, 2020) we assessed relationships between repeatable behavioral variables using mutual information analysis (MI; Pardy and Wilson, 2011) separately for each age. MI quantifies the reduction in uncertainty (entropy) of the value of one variable given the knowledge of another, and it can be used to examine nonlinear/non-monotonic relationships. Only the behaviors showing repeatable individual differences at each age group (agreement and/or adjusted repeatability; Tables 2 and 3) were included in this analysis. To examine only among-individual level associations between behaviors, each subject's mean value of a given behavior at each age was used, thus eliminating within-individual variation

(Dingemanse et al., 2012). Each possible pair of repeatable behaviors at each age was analyzed using jackknife-bias corrected mutual information (BCMI), calculated using the *mpmi* R package (Pardy, 2019). *P*-values were calculated using 1,000 permutations, and the critical α -value was adjusted for false discovery rate according to Benjamini and Hochberg (1995).

3 RESULTS

3.1. Repeatability of behaviors

In the struggle/ handling and separation/ confinement tests, the behaviors measured were repeatable (both agreement and adjusted repeatability) in all age groups (Tables 2 and 3). In the mouse test, the time spent near the jar was repeatable from 6 months onwards, and time spent sniffing the mouse jar was repeatable from 12 months onwards. In the meat test, the reduced frequency of the behaviors combined with the reduced sample size from the ages of 6 months onwards impeded the reliable assessment of repeatability and its associated CI and *P*-value. At 2 months of age, the kittens stomped or swiped in 35.4% of the trials; however, the frequency of this behavior declined with age (6 months: 14.9%; 12 months: 16.4%; 18 months: 0%). Similarly, at 2 months of age kittens carried away the meat in only 11.8% of the trials (but the larger sample size made repeatability calculations possible), which increased to 28.2% at 6 months but declined thereafter (12 months: 14.8%, 18 months: 11.3%).

In the struggle/ handling, mouse, and separation/ confinement tests, within-age (short-term) agreement and adjusted repeatability of behaviors tended to increase with age (Tables 2 and 3; Figure 1). In these tests, within-individual (residual) variance tended to decrease with age, and for all but one behavioral variable (number of meows), among-individual variance generally

tended to increase with age (Supplementary Material Table 2, Figure 1). It's important to note, however, that neither within- nor among-individual variance showed completely linear nor constant patterns of change across age groups; rather, these were long-term trends (Figure 1).

In the struggle/ handling, mouse, and separation/ confinement tests, the repeatability of behavioral variables across development (inclusive of all age groups) tended to be comparatively low (Tables 2, 3). However, behavior was stable between consecutive age groups; it was the least stable (though still significantly repeatable) between 2 and 6 months of age, and repeatability steadily increased between later age groups pairings (Table 5).

3.2. Behavioral syndromes

At the ages of 2, 6, 12 and 18 months, no pairs of behaviors had a significant BCMI value, indicating no associations between behaviors. Thus, there was no evidence of behavioral syndromes in any age group.

4 DISCUSSION

In our previous research, we found stable (repeatable) inter-individual differences in behavior in a series of ecologically relevant test situations in adult domestic cats housed in a shelter environment (Urrutia et al. 2019, Martínez-Byer et al., 2020), and in 2-month-old domestic kittens in a private home (Urrutia et al., 2022). In the present report, we followed a subset of the kittens from the latter study and evaluated their behavioral stability within and between life stages by repeatedly testing them at 2, 6, 12 and 18 months of age.

Consistent with our previous work, we found several repeatable behaviors in every age group, indicating stable inter-individual differences or personality (Tables 2 and 3). In addition, we found that repeatability generally was higher in older age groups (Tables 2 and 3). High repeatability is driven by low within-individual variance (i.e. by individuals behaving consistently), and/or high among-individual variance (i.e. individuals behaving differently from each other). An examination of the variance components of the behaviors showed that within-individual variance tended to decrease with age, and among-individual variance generally tended to increase with age for all but one behavioral variable (Supplementary Material Table 2), which together resulted in the observed trend of increased repeatability with age. There are at least three mechanisms which could potentially drive these changes in variance: canalization, developmental plasticity and state-behavior feedback (reviewed in Kok et al., 2019), which we discuss below.

The age-related decreases in within-individual variance found in this study are consistent with previous findings in mosquitofish *Gambusia holbrooki* (Polverino et al., 2016), painted turtles *Chrysemys picta* (Delaney et al., 2020), great tits *Parus major* (Thys et al., 2021), red knots *Calidris canutus* (Kok et al., 2019), and red squirrels *Tamiasciurus hudsonicus* (Martinig et al., 2021). The cats of the present study faced such stable conditions: they had reliable access to food, were kept in good health, and from 6 months of age onwards they generally remained in the same home environment and interacted with the same people. When organisms face stable conditions resulting in predictable environmental cues, stabilizing trait expression (canalization) can occur, favoring stability of behavior (Kok et al., 2019). At a mechanistic level, the neural and endocrine systems that underlie behavior can be slow and energetically costly to change in adulthood, favoring stability of behavior in later life stages (Coppens et al., 2010; Duckworth, 2010).

With regard to the increases in among-individual variance, developmental plasticity can encourage divergence (a ‘fanning out’) in among-individual behavior over ontogeny due to

feedback mechanisms which, over time, act on differences that were present early in life (Stamps and Biro, 2016). We tested for two aspects of early life that in other species have been found to affect behavior at later ages: birth mass (e.g. Rödel & Meyer, 2011) and litter size (e.g. Dimitsantos et al., 2007), however they had null or negligible effects on behavior in the present study. There are other early life differences which can affect developmental plasticity, for example litter sex ratio (Mendl & Paul, 1990) or maternal effects (Taylor et al., 2012) which include not only genetic and epigenetic effects, but also other aspects such as rearing behaviors. However, the small number of litters (16) and mothers (6) which were not equally represented throughout the different age groups of our study did not allow for the exploration of their related early life effects on personality in later ages, and these questions require further research.

The increases in among-individual variance could also be attributed to differences in the cats' post-weaning conditions or environments creating state-behavior feedback loops, which fix individuals on different trajectories (Sih et al., 2015). In general, we consider that the cats were housed under relatively homogeneous conditions: they were all born into the same private home, maintained good health, were kept as companion animals, and had positive interactions with humans; they did not face important environmental stressors such as food shortage or risk of predation at any stage, which can be important sources of individual variation in other species (Stamps and Krishnan, 2014a). One potentially important difference in their post-weaning environments was whether or not they had access to outdoor spaces such as gardens. Specifically, we wondered if this could have affected the outcome of the mouse test: if indoor-outdoor cats gained more hunting experience than indoor-only cats, this could increase among-individual variation in the behaviors expressed in the mouse test. However, we did not find any effect of housing conditions on the behavior in this test. Of course, although the cats' home environments all shared the general characteristics mentioned above, even extremely small and unaccounted for

differences in condition and environment can act as sources of variation which lead to state-behavior feedback (Bierbach et al., 2017), and we cannot fully discount these effects.

Despite the short-term repeatability of many behaviors in several age classes, we observed only moderate or non-significant repeatability of behaviors across the entire age span of the study, 2-18 months of age, even when including age class as a fixed effect (Tables 2, 3). However, pairwise comparisons between consecutive age groups (2-6, 6-12 and 12-18 months) yielded comparatively higher between-age repeatability, particularly between later ages (Table 4). This suggests that the comparatively low long-term repeatability (spanning all age classes) was not due to sudden developmental changes overriding early individual differences during any single identifiable period of major behavioral reorganization, but rather that individuals' behaviors gradually changed over a longer time span, becoming more stable later in life (Table 4). This finding supports previous reports that repeatability is higher when between-test intervals are shorter (Bell et al., 2009; Stamps and Krishnan, 2014b).

In the case of the meat test, we have previously reported low but significant repeatability of stomping/swiping at the tongs and of carrying the meat away from the experimenter at the age of 2 months (Urrutia et al., 2022). From 6 months onwards, however, we were not able to reliably calculate these repeatabilities due to the reduced sample sizes combined with the low frequency at which the behaviors occurred. In adult cats, food-defense related agonistic behaviors directed towards conspecifics have been reported to show individual consistency, and they appear to contribute to the maintenance of stable feeding orders or hierarchies in groups of cats (Knowles et al., 2004; Bonanni et al., 2007). In the case of the companion cats of the present study, they may have learned not to direct stomping and swiping towards humans by the time they were juveniles and adults; this could explain the reduced occurrence of these behaviors, which fell from 35.4% of trials at 2 months to 14.9% of trials at 6 months, and eventually their complete absence at 18

months. In addition, while 2-month-old kittens often hissed or growled during the meat test (Urrutia et al., 2022), we did not observe this behavior in any other age group in the present study. Therefore, food-defense related behaviors may be best studied when they are directed towards a conspecific rather than an experimenter or an object. With this caveat, these behaviors remain an interesting aspect of individual differences in behavior which can have a direct impact on fitness (Bonanni et al., 2007).

We did not find evidence of behavioral syndromes at any stage during the development of the domestic cat, i.e. repeatable behaviors at all ages lacked associations amongst each other. This is in contrast to our previous findings in adult cats housed in a shelter environment, where behavioral syndromes were identified using three of the same tests (struggle/handling, mouse, and separation/confinement; Martínez-Byer et al., 2020). In the aforementioned study, behavioral syndromes appeared to be mainly due to human-oriented behaviors. The cats that participated in that study were a heterogeneous sample that differed in their backgrounds: the amount and type of experience they had with humans prior to their arrival at the animal shelter was diverse and largely unknown. While the shelter cats from Martínez-Byer et al. (2020) all tolerated at least the brief handling by experimenters required for the tests, their demeanor ranged from extremely human-oriented to mildly human-averse (own observations). In contrast, the cats included in the present study were all handled by the experimenters from a very early age and continued to be handled regularly by their new owners after being rehomed, and readily permitted handling by the experimenter during the visits to their homes. Early handling in particular positively influences cats' behavior towards humans even years later (see review by Turner, 2017), contributing to the marked differences in behavior between feral and house cats (Yeon et al., 2011). Including a mix of human-oriented and human-averse cats in a study could increase among-individual variance in the behaviors measured. We hypothesize that because the cats of the present report were more

homogeneous in their relationship with humans than the shelter cats studied in Martínez-Byer et al. (2020), there was not enough variation in the present study to observe the previously described behavioral syndrome. It has been reported in other species that the structure of behavioral syndromes can vary between populations that live in differing conditions (e.g. Dingemans et al., 2007; Evans et al., 2010; Michelangeli et al., 2019), and we propose that the domestic cat, with its worldwide distribution across different environments and lifestyles which span from companion animals to fully feral cats, could be a good model for further research on this topic.

Despite the wealth of animal personality studies conducted on adult animals, there are still relatively few that focus on development (Cabrera et al., 2021). Here we show evidence that individual differences in behavior in the domestic cat are present from a very early age, and become increasingly repeatable throughout development and into adult life. This is due to a combination of decreased individual-level variance (canalization) and increased among-individual variance in behavior. In addition, we did not observe behavioral syndromes, in contrast to previous results in a different population of cats. Potential topics for further research in this area include the mechanisms that drive the increased repeatability with age and the differences in personality structure between populations in this species.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

ORCID

Andrea Urrutia <https://orcid.org/0000-0001-5936-9740>

Oxána Bánszegi <https://orcid.org/0000-0002-1704-3539>

Péter Szenczi <https://orcid.org/0000-0002-8495-1633>

Robyn Hudson <https://orcid.org/0000-0002-5526-9555>

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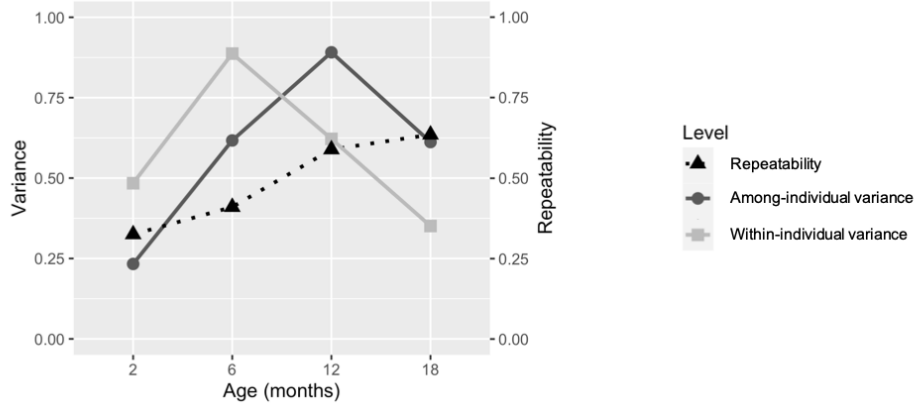
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Figure legends

Figure 1. Variance components and agreement repeatability of behaviors. Aligned with the left y-axis, dark gray lines denote among-individual variance and light gray lines, within-individual variance; aligned with the right y-axis, dashed black lines denote repeatability.

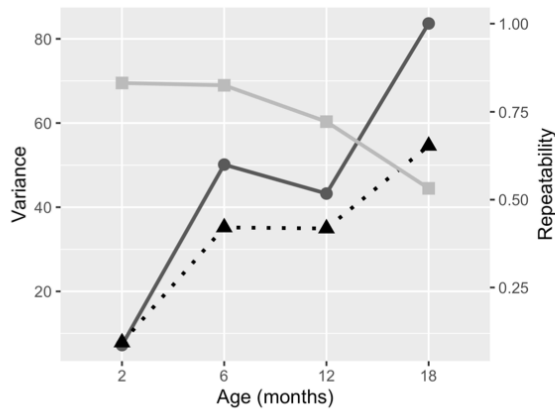
Struggle test

Latency to struggle

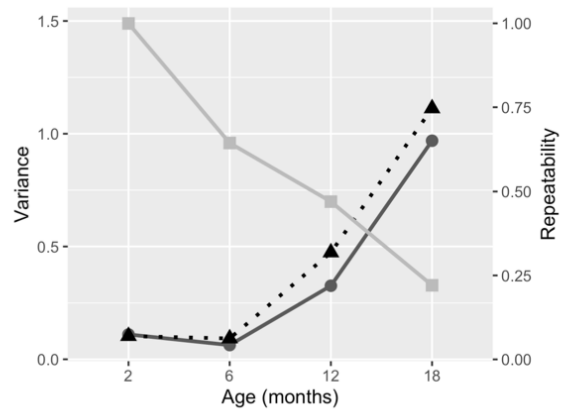


Mouse test

Time near the mouse

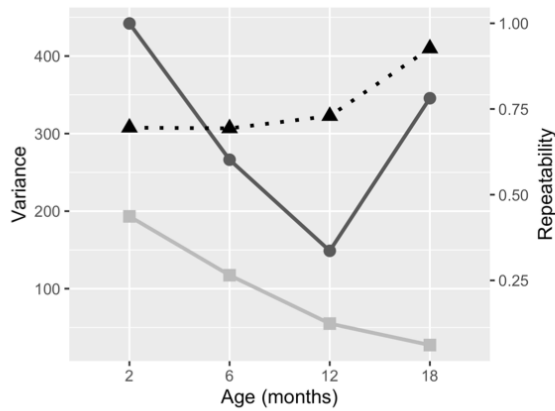


Time sniffing the mouse



Separation/confinement test

Number of meows



Duration of motor activity

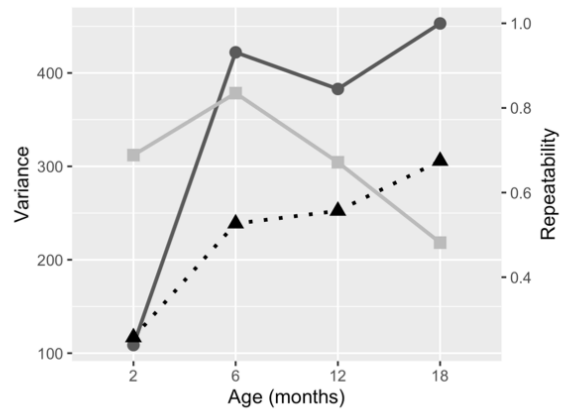


Table 1

Behavioral variables analysed in each test.

Behavior measured	Definition
Struggle / handling test	
Struggle (latency, s)	Lifting one of the hind paws and touching or kicking the experimenter's forearm
Meat test	
Stomp or swipe (binary)	If the cat stomped on the floor or tongs with a forepaw and/or swiped at the tongs at least once
Carry away (binary)	If the cat walked or ran away (>3 steps) from the experimenter, carrying the meat at any point during the test
Mouse test	
Near the mouse (duration, s)	When at least the cat's forepaws were within one body length of the jar
Sniffing the jar (duration, s)	Sniffing the jar
Separation / confinement test	
Meows (number)	Meow-type vocalizations (Nicastro, 2004)
Motor activity (duration, s)	Displacement of any of the limbs on the floor or sides of the carrier for at least 1 s

(Urrutia et al.)

Table 2

Agreement repeatabilities (R) and 95% confidence interval [95% CI] of the measured behavioral variables for each test. Bolded values are significant ($P < 0.05$ and the 95% CI does not include 0). Only individual identity was included as a random factor for within-age group comparisons; note that for between-age group repeatability, age group was added as a fixed effect.

Outcome variable	2 months	6 months	12 months	18 months	All ages
Struggle/ handling test					
Latency to struggle	0.325 [0.152, 0.47]	0.41 [0.17, 0.596]	0.589 [0.325, 0.764]	0.635 [0.295, 0.819]	0.211 [0.104, 0.311]
Mouse test					
Duration near the mouse	0.094 [0, 0.249]	0.421 [0.176, 0.596]	0.418 [0.13, 0.643]	0.653 [0.311, 0.835]	0.195 [0.096, 0.298]
Duration sniffing the mouse	0.069 [0, 0.243]	0.062 [0, 0.256]	0.318 [0.023, 0.568]	0.747 [0.46, 0.887]	0.09 [0.01, 0.173]
Separation/ confinement test					
Number of meows	0.696 [0.563, 0.788]	0.694 [0.528, 0.803]	0.73 [0.497, 0.847]	0.927 [0.837, 0.967]	0.498 [0.372, 0.6]
Motor activity	0.258 [0.073, 0.42]	0.527 [0.311, 0.675]	0.557 [0.266, 0.745]	0.675 [0.337, 0.841]	0.292 [0.169, 0.407]

(Urrutia et al.)

Table 3

Adjusted repeatabilities (R) and 95% confidence interval [95% CI] of the measured behavioral variables for each test at each age class. Bolded values are significant ($P < 0.05$ and the 95% CI does not include 0). Individual identity was fitted as a random factor and the covariates listed were included in the models as fixed effects; note that where age or age*sex are listed as covariates, they were included only in the model that included all age classes and excluded from the models that included data from single age classes.

Outcome variable	Covariates	2 months	6 months	12 months	18 months	All ages
Struggle/ handling test						
Latency to struggle	Age + trial	0.37 [0.212, 0.525]	0.451 [0.245, 0.638]	0.645 [0.4, 0.8]	0.639 [0.313, 0.823]	0.22 [0.111, 0.326]
Mouse test						
Duration near the mouse	Age	0.12 [0, 0.277]	0.375 [0.149, 0.559]	0.422 [0.133, 0.625]	0.651 [0.315, 0.841]	0.167 [0.074, 0.258]
Duration sniffing the mouse	Age	0.094 [0, 0.238]	0.038 [0, 0.252]	0.337 [0.048, 0.587]	0.762 [0.521, 0.881]	0.074 [0.003, 0.155]
Separation/ confinement test						
Number of meows	Age + sex + age*sex	0.574 [0.419, 0.689]	0.729 [0.563, 0.835]	0.672 [0.422, 0.817]	0.928 [0.855, 0.968]	0.457 [0.336, 0.563]
Motor activity	Age + trial	0.411 [0.242, 0.552]	0.509 [0.305, 0.667]	0.671 [0.459, 0.817]	0.681 [0.42, 0.849]	0.321 [0.211, 0.427]

(Urrutia et al.)

Table 4

Between-age agreement repeatabilities (R) and 95% confidence interval [95% CI] of the measured behavioral variables for each test. Bolded values are significant ($P < 0.05$ and the 95% CI does not include 0). Only individual identity was included as a random factor.

Outcome variable	2-6 months	6-12 months	12-18 months
Struggle/ handling test			
Latency to struggle	0.237 [0.119, 0.367]	0.262 [0.101, 0.41]	0.436 [0.206, 0.618]
Mouse test			
Duration near the mouse	0.132 [0.025, 0.248]	0.398 [0.221, 0.546]	0.463 [0.237, 0.635]
Duration sniffing the mouse	0.07 [0, 0.166]	0.141 [0.002, 0.28]	0.337 [0.048, 0.587]
Separation/ confinement test			
Number of meows	0.475 [0.355, 0.573]	0.643 [0.477, 0.757]	0.735 [0.57, 0.84]
Motor activity	0.281 [0.154, 0.392]	0.43 [0.259, 0.578]	0.532 [0.317, 0.689]

(Urrutia et al.)

Supplementary Material

Table 1

Number of individuals, litters, and trials for which data were available for each test in each age group.

Test	Age (months)	Individuals tested (litters)	Trials recorded
Struggle/ handling	2	70 (15)	191
	6	40 (15)	105
	12	27 (13)	69
	18	19 (12)	46
Meat	2	72 (16)	177
	6	39 (15)	104
	12	26 (12)	61
	18	18 (11)	44
Mouse	2	74 (16)	195
	6	38 (15)	103
	12	28 (13)	70
	18	18 (11)	43
Separation/ confinement	2	74 (16)	184
	6	39 (15)	105
	12	26 (12)	67
	18	18 (11)	45

(Urrutia et al.)

Table 2

Repeatability and variance components of agreement repeatabilities of the measured behavioral variables for each test. Among: among-individual variance, Within: within-individual variance, Repeatability: agreement repeatability.

Behavioral variable		2 months	6 months	12 months	18 months
Struggle/ handling test					
Latency to struggle	Among	0.233	0.617	0.891	0.612
	Within	0.484	0.887	0.622	0.351
	Repeatability	0.325	0.41	0.589	0.635
Mouse test					
Duration near the mouse	Among	7.247	50.123	43.247	83.659
	Within	69.517	68.964	60.325	44.478
	Repeatability	0.094	0.421	0.418	0.653
Duration sniffing the mouse	Among	0.11	0.063	0.326	0.969
	Within	1.489	0.959	0.699	0.328
	Repeatability	0.069	0.062	0.318	0.747
Separation/ confinement test					
Number of meows	Among	442.046	266.303	148.844	345.605
	Within	193.265	117.374	54.999	27.312
	Repeatability	0.696	0.694	0.73	0.927
Motor activity	Among	108.678	422.115	382.855	453.031
	Within	312.077	378.434	304.391	218.345
	Repeatability	0.258	0.527	0.557	0.675

(Urrutia et al.)

Capítulo IV

Evaluación de las diferencias individuales en la respuesta a un estresor agudo cotidiano, durante el desarrollo en el gato doméstico

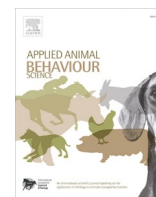
Scaredy-cat: Assessment of individual differences in response to an acute everyday stressor across development in the domestic cat

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Scaredy-cat: Assessment of individual differences in response to an acute everyday stressor across development in the domestic cat

Andrea Urrutia ^{a,b,c,1,*}, Oxána Bánszegi ^{b,2}, Péter Szenczi ^{c,3}, Robyn Hudson ^{b,4,**}

^a Posgrado en Ciencias Biológicas, Unidad de Posgrado, Edificio A, 1er Piso, Circuito de Posgrados, Ciudad Universitaria, Coyoacán, CP 04510 Mexico City, Mexico

^b Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, AP 70228, CP 04510 Mexico City, Mexico

^c CONACYT - Instituto Nacional de Psiquiatría Ramón de la Fuente Muñiz, Unidad Psicopatología y Desarrollo, Calz. México-Xochimilco 101, CP 14370 Ciudad de México, Mexico

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ABSTRACT

A concern in animal welfare is the degree to which the animals in our charge experience stress during everyday procedures. Central to this is how to appropriately assess stress in relevant daily contexts. As one of the most popular companion animals worldwide, the domestic cat *Felis silvestris catus* is a good example. The cat may experience situations in daily life which can be considered stressful, such as confinement in a pet carrier for veterinary visits or to accompany its owners on holiday. It was therefore our aim in the present study to assess the response of pet domestic cats to brief confinement in a standard pet carrier during which we employed two behavioural and one non-invasive physiological measure thought to be indicators of stress. We investigated the presence of individual differences in 74 kittens' responses before weaning (before 2 months of age) and the stability of these across development to adulthood in a subset of the same individuals tested after adoption in their new homes at 6, 12 and 18 months of age. Individuals were tested by placing them alone for 2 min in a pet carrier in an isolated room. Motor activity and vocalisation were continuously recorded, and eye and nose temperature were registered immediately before and after each test using a thermal camera. Statistical analyses showed stable (repeatable) individual differences for various measures of vocalisation and to a lesser extent for motor activity within and across age classes, but no relation between measures of the two. Thermographic measures of eye temperature showed no consistent pattern of thermal response to the test, and only weak and contradictory patterns of change in the temperature of the nose. In conclusion, we suggest measures of vocalisation to be a good indicator of individual differences of stress in response to such everyday procedures in the cat, although still needing validation from physiological measures. Facial thermography presently does not seem sufficiently reliable in this regard. Additionally, we emphasize the importance of conducting repeated tests across time to identify and develop reliable indicators of stress in whatever species.

1. Introduction

Stress can be defined biologically as “a cognitive perception of uncontrollability and/or unpredictability that is expressed in a physiological and behavioural response” aimed at coping with the stimulus

that causes it, called a “stressor” (Koolhaas et al., 2011). “Coping styles” encompass the behavioural and physiological response to stressors in which there is individual consistency across time (Koolhaas et al., 1999). During the stress response, the sympathetic nervous system and Hypothalamic-Pituitary-Adrenal (HPA) axis release the catecholamine

* Corresponding author at: Posgrado en Ciencias Biológicas, Unidad de Posgrado, Edificio A, 1er Piso, Circuito de Posgrados, Ciudad Universitaria, Coyoacán, CP 04510 Mexico City, Mexico.

** Corresponding author.

E-mail addresses: andreaurrutia@outlook.com (A. Urrutia), oxana.banszegi@gmail.com (O. Bánszegi), peter.szenczi@gmail.com (P. Szenczi), rhudson@biomedicas.unam.mx (R. Hudson).

¹ <https://orcid.org/0000-0001-5936-9740>

² <https://orcid.org/0000-0002-1704-3539>

³ <https://orcid.org/0000-0002-8495-1633>

⁴ <https://orcid.org/0000-0002-5526-9555>

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hormone epinephrine from the adrenal medulla, and glucocorticoid hormones from the adrenal cortex, which have a wide range of effects on the body (Romero and Butler, 2008). While stress is adaptive in the wild and allows animals to respond appropriately to threatening or dangerous situations, in domestic animals, captive wild animals and humans prolonged or chronic stress can have serious negative effects on the immune system, metabolism, reproduction and cognition (Sapolsky et al., 2000). In the case of domestic animals, prolonged or chronic stress can also lead to poor welfare which results in economic loss in the case of farmed animals due to its negative effects on growth, reproduction, milk yield and meat quality (Grandin and Shivley, 2015) and in negative health outcomes and undesirable behaviours in companion animals (e.g. Amat et al., 2015). Therefore, it is important to have reliable indicators of stress. In addition, according to the concept of coping styles, good indicators should be repeatable across time, and should be consistent with each other.

At the behavioural level, components of the “fight or flight” response are often used as indicators of distress, for example, increased or reduced locomotion (“freezing”), vocalisation or alertness, with the exact behaviours depending on the species and the stressor. Vocalisation has been proposed as an indicator of emotion (Briefer, 2012); vocalisation rate and/or quality have been found to reflect distress in a variety of species (e.g. domestic cattle: Bristow and Holmes, 2007; horses: Pond et al., 2010; domestic pigs: Schrader and Todt, 1998; domestic cat: Brown et al., 1978), and can be used to monitor farm animals in real-time for signs of stress (Moura et al., 2008). However, it is important to note that although behaviour may seem simple and quick to elicit and observe, its control is complex and it can have multiple underlying motivations and mechanisms which complicate its interpretation (Rushen, 2000). Therefore, where possible, behavioural indicators of stress should be studied in conjunction with physiological indicators, and the tests and stressors used to elicit them should be carefully chosen (e.g. Van Reenen et al., 2004, 2005, 2013).

At the physiological level, in recent years infrared thermography (IRT) has seen a rise in popularity as a tool to assess emotion in animals (particularly in response to acute stressors) and the activation of the autonomic nervous system (see review by Travain and Valsecchi, 2021). This assessment is achieved by detecting changes in peripheral body temperature due to changes in blood flow; during stress, sympathetically mediated vasodilation and vasoconstriction play important roles in modifying temperature in different body areas, causing increases and decreases in temperature, respectively (Travain and Valsecchi, 2021). IRT is advantageous because it is a non-invasive technique and it provides real-time measurements. It has therefore been explored as a possible tool for evaluating welfare, mainly in domestic animals in production, companion animals, laboratory animals, working animals in various contexts, and animals in sport (e.g. Godyń et al., 2013; Travain et al., 2015; Kim and Cho, 2021; Mota-Rojas et al., 2021).

An important part of any study that aims to evaluate individual differences in coping styles is the selection of species-appropriate, biologically relevant tests which can reliably elicit a stress response. Common, simple and ethically permissible tests are various forms of social isolation and/or confinement. In domestic animals, isolation and/or confinement are highly relevant situations that are part of the normal management of both livestock and companion animals. Isolation/confinement tests have been previously used to identify stable individual differences in behaviour in adult domestic cats, *Felis silvestris catus* (Iki et al., 2011; Yeon et al., 2011; Urrutia et al., 2019; Martínez-Byer et al., 2020) and kittens (Hudson et al., 2015, 2017; Urrutia et al., 2022). Brief isolation/confinement is stressful and may elicit distress calls in cats of all ages (Brown et al., 1978; Nicastro, 2004; Schötz et al., 2019), and is also accompanied by changes in core body temperature (Marchei et al., 2009). Thus, in the domestic cat, isolation/confinement tests are a useful tool to assess differences in individuals’ reactions to acute stress.

In this study we had two main aims. First, to investigate the short- and long-term consistency of individual differences in three different

indicators of the behavioural and physiological response to an acute stressor in a domestic mammal –the domestic cat– across development and into adult life. And second, to investigate the association among the three responses in order to assess their usefulness in combination as indicators of individual differences in the response to an everyday stressor across development.

2. Animals, materials and methods

2.1. Study site and animals

From April 2016 to October 2017, we tested 74 mixed-breed kittens (36 males, 38 females) from 16 litters (mean litter size 4.7 ± 1.4 SD, range 2 – 7) born to six mothers (mean number of kittens per mother 12.5 ± 5.4 SD) maintained as part of a breeding colony at a private house in Mexico City. The colony females were free to leave and enter the house at will and to mate with roaming local males, hence paternity of the kittens was unknown (multiple paternity is common in domestic cats; Say et al., 1999). All mothers gave birth voluntarily in the house. Mothers showed little interest in the litters of other females, and communal nursing did not occur. Each litter was housed in a separate room (approximately 3 m × 3 m) of the house. The rooms had doors 1.2 m in height so that the mothers could jump freely in and out, but the kittens were unable to leave. Newborn litters were provided with a commercial foam pet bed placed inside a large open-top cardboard box (60 × 80 × 70 cm) with a small opening cut for the mother at floor level. The box was removed when the kittens were 4 weeks old and began leaving the nest bed, at which time various enrichment objects, a scratching board, and a sand box were placed in their room. From this time, kittens had permanent access to dry commercial cat food and water, and were also provided daily with commercial canned cat food. Kittens were weighed at birth and daily thereafter to the nearest gram using digital scales to check for adequate weight gain and to habituate them to human handling. Their sex was registered, and each was fitted with a different colour neck ribbon for individual identification.

Starting at 5 weeks of age, each kitten was tested three times in a separation/isolation test as described in Section 2.2 below. At 8 weeks of age the kittens were given away as pets to people living in the Mexico City Metropolitan Area. All cats were neutered soon after adoption except for one male and one female. However, as removing them from the analysis had no effect on the results, we retained their data to maximize sample sizes.

A subset of these cats was later visited in their new homes at the ages of 6, 12, and 18 months (see Table 1 for details of sample sizes). For these cats, at each age, three visits to their homes were arranged, leaving 1–6 days between visits. On each visit, the test was repeated as described in Section 2.2 below. Due to technical problems resulting in data loss, some data from some trials are missing, and additionally, not all cats in the older age groups were available for all trials. In these cases, analyses

Table 1

Number of individuals and trials for which video (V), audio (A), and thermal (IRT) data were available for each age group.

	V	A	IRT
Pre-weaning			
Individuals tested	74	56	58
Trials recorded	211	146	176
6 months			
Individuals tested	40	37	40
Trials recorded	109	91	107
12 months			
Individuals tested	27	23	28
Trials recorded	71	59	72
18 months			
Individuals tested	21	14	17
Trials recorded	51	50	43

(Urrutia et al.)

were carried out with the data available (Table 1).

2.2. Test procedure

For the pre-weaning age kittens, on postnatal weeks 5, 6 and 7, and in the absence of their mother and littermates, each individual was tested in a brief separation/confinement test by an experimenter with whom it was familiar. Testing took place in an unfamiliar room (2.4 × 0.9 m) to which the cats in the colony did not otherwise have access, and which was on a different level of the house to the litters' home rooms to minimize auditory and olfactory cueing. Littermates were tested in random order, determined by lottery cards marked with the kittens' identities. While the rest of the litter remained in its home room, the experimenter carried the focal kitten to the test room. Immediately before the start of the test, three to four baseline thermal photographs (Fluke Ti25, Fluke Corporations, Seattle, USA) were taken of the kitten's face. The pictures were taken from a distance of ~30 cm and the experimenter positioned the kitten to directly face the camera by placing it on a flat surface and gently scruffing its neck. The thermal photography process generally took a maximum of 1 min.

After obtaining the baseline thermal images, the kitten was moved to a commercial pet carrier (38 × 58 × 30 cm) with a metal grill door, which was placed on the floor of the test room. The interior of the carrier was fitted with two LED strips to improve visibility for later video analysis, and the interior of the carrier was wiped down with isopropyl alcohol prior to testing. A video camera (Sony HDR-CX130), and a sound recorder (Tascam DR-40, Montebello CA; 96 kHz / 24 bit) were set up 60 cm from the door of the carrier and directed towards it. The experimenter began recording on the video and sound recorders, locked the kitten in the carrier and left the room, at which point the test began. After the kitten had remained alone in the room for 2 min, the test ended, the experimenter retrieved the kitten and removed it from the carrier, took three to four additional thermal photographs following the same procedure described above, and took the kitten back to its home room.

After weaning and adoption, the same procedure was followed using the same carrier in an isolated room of the cats' new homes, and the test was recorded in the same way. During our visits to their homes, these older cats participated in three other behavioural tests prior to the isolation/confinement test. These other tests were designed to investigate different characteristics of the cats' behaviour and are not thought to be stressful; these tests were: a brief handling test; a test in which a piece of beef was given to, and then removed from, the cat; and a test in which the cat was presented with a live mouse in a glass jar (sealed, with a perforated lid). The results of these other tests will be reported in a separate paper. This testing order was maintained throughout the study. The cats were given a 5–10 min pause between the last of these tests and the start of the isolation/confinement test.

2.3. Data processing and statistical analysis

2.3.1. Behavioural data

The duration of motor activity (displacement of any of the limbs on the floor or sides of the carrier; quantified in seconds at intervals of 0.5 s by analysis of video footage for each individual in each trial) was coded from the videos using Solomon Coder software (Péter, 2011), as described in previous work (Urrutia et al., 2019, 2022).

Vocalisations that were free of background noise were identified from the sound recordings of the tests using the software Praat version 6.1.09 (Boersma and Weenink, 2021) (Fig. 1). Each vocalisation was then copied to a separate file and analyzed using the Python library Parselmouth (Jadoul et al., 2018), which interfaces Praat. Pitch was set to 300–1500 Hz (Brown et al., 1978). For each trial, the number of vocalisations was noted, and the mean duration (s), mean fundamental frequency (F_0 , Hz) and mean standard deviation of the fundamental frequency ($SD F_0$) of each vocalisation were calculated (Briefer, 2012)

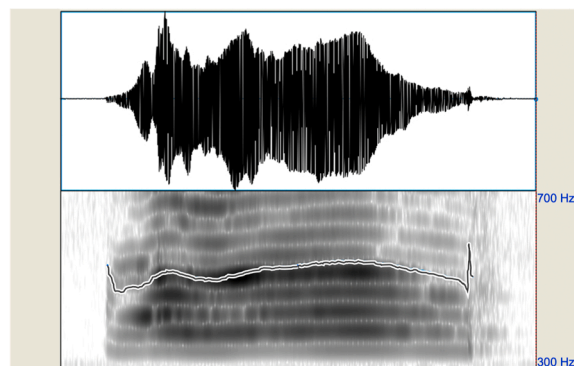


Fig. 1. Oscillogram (top) and spectrogram (bottom) obtained using Praat software of a meow-type vocalisation (Nicastro, 2004) produced by a 12-month-old female cat during an isolation/confinement trial. The fundamental frequency (F_0 , highlighted line) is shown in the spectrogram. The window duration is 1 s.

Table 2

Definition of the variables measured in the tests.

Variable measured	Definition
Motor activity (duration, s)	Displacement of any of the limbs on the floor or sides of the carrier for at least 1 s
Number of vocalisations	Number of vocalisations
Mean duration (s) of vocalisations	Mean duration of each vocalization emitted in a trial
Mean fundamental frequency (F_0) of vocalisations	Mean F_0 (calculated for each vocalisation, and averaged across all vocalisations in a trial)
Mean standard deviation of the fundamental frequency ($SD F_0$) of vocalisations	Standard deviation from the mean F_0 (calculated for each vocalisation, and averaged across all vocalisations in a trial)
Change in eye temperature ($^{\circ}\text{C}$)	Difference between the temperature of the hottest part of the medial canthus, in $^{\circ}\text{C}$, before and after each trial. This was calculated separately for each eye (Fig. 2)
Change in nose temperature ($^{\circ}\text{C}$)	Difference between the temperature of the coldest part of the external surface of the nose in $^{\circ}\text{C}$, before and after each trial (Fig. 2)

(Urrutia et al.)

(Table 2; Fig. 1).

2.3.2. Thermal data

We used SmartView thermal imaging software version 4.3.329.0 (Fluke Corp., Everett, Washington, USA) to analyze images (Fig. 2). The maximum temperature of the medial canthus (caruncula) of each eye was registered. This is the hottest point of the eye and is thought to be the most representative of core body temperature and has been used previously in stress studies in a range of mammalian species (see review by Travain and Valsecchi, 2021). Additionally, the minimum temperature of the nose was registered. From the three to four images collected, the mean temperature of each region was obtained, before and after each test. We then calculated the change in $^{\circ}\text{C}$ before and after the test in the maximum medial canthus temperature of each eye, and the change in the minimum temperature of the nose. Since the temperature changes in the right and left eye were highly correlated (repeated measures correlation $r_{rm} = 0.761$, $P < 0.0001$; Bakdash and Marusich, 2018), and there was no significant difference between them (paired t test: $t_{385} = -0.258$, $P = 0.79$), the mean temperature change of both eyes was used for the statistical analyses.

2.3.3. Statistical analysis

Data analysis was carried out using the programme R, version 4.1.2 (R Core Team, 2021).

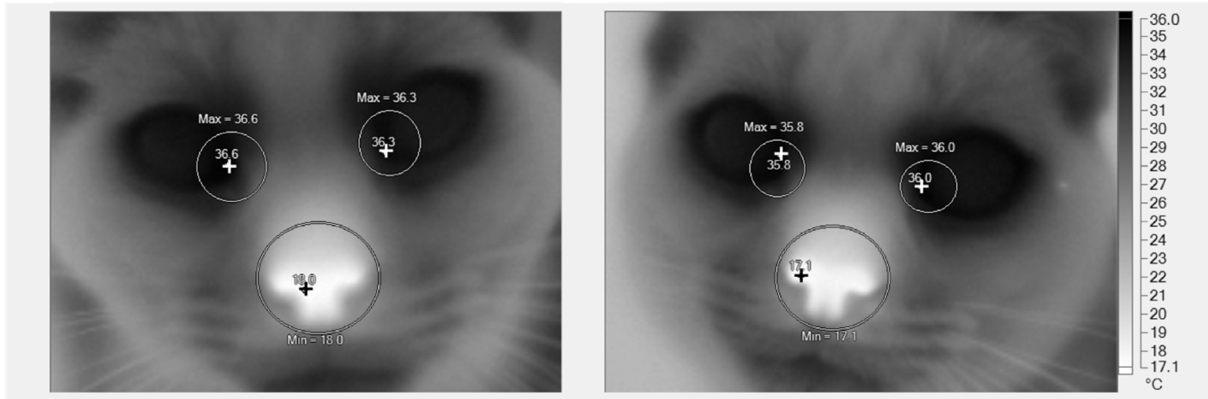


Fig. 2. Infrared image of the face of a 12-month-old female cat immediately before (left) and after (right) the isolation/confinement test. The circles indicate the regions of interest, from which the maximum temperature of each canthus of the eyes and the minimum temperature of the nose (indicated with cross hairs) were registered.

We first checked for age-related change in each variable (behaviour: duration of motor activity, number of vocalisations, mean vocalisation duration, mean vocalisation F_0 , mean vocalisation $SD F_0$; IRT: temperature change of the eyes' medial canthi, temperature change of the nose). Data were averaged by individual and age group, and age groups were compared using Wilcoxon matched pairs tests; P -values were adjusted for multiple comparisons according to Benjamini and Hochberg (1995).

We tested for significant individual and maternal effects (fitted as random factors) and effects of trial (1–3), sex, age and age*sex (fitted as fixed factors) for each of the outcome variables by fitting linear mixed models (LMMs) using the R package *lme4* (Bates et al., 2015). P -values for fixed effects were obtained using the R package *lmerTest* (Kuznetsova et al., 2017). In the models predicting temperature change of the eyes and of the nose we also included the baseline temperature of the respective area as a fixed effect to account for variables which could affect pre-test temperature (time of day, ambient temperature; Jerem et al., 2019). Plots of residuals versus fitted values and Q-Q plots were used to visually inspect model residuals for homogeneity of variance and normal distribution. Models were reduced using backwards stepwise selection while retaining individual identity in the models.

Repeatability (the amount of variance explained by the individual divided by the total phenotypic variance; Sokal and Rohlf, 1995), of each variable (behaviour: duration of motor activity, number of vocalisations, mean vocalisation duration, mean vocalisation F_0 , mean vocalisation $SD F_0$; IRT: temperature change of the eyes' medial canthi, temperature change of the nose) were calculated using intra-class correlations obtained through LMM-based calculations using the R package *rptR* (Nakagawa and Schielzeth, 2010). Repeatability was calculated in two ways: first, agreement repeatability, where only the individual identity was included as a random factor, and no fixed effects were included; and second, adjusted repeatability, where individual identity was included as a random factor, and any additional fixed or random effects found to have a significant effect in the LMMs described above were included where applicable (Nakagawa and Schielzeth, 2010). P -values were calculated using 1000 permutations, and 95% confidence intervals (95% CI) for parameter estimates were assessed from 1000 bootstrapping runs.

We then tested for associations between the variables which were found to be repeatable in the previous analysis. The data were treated in two ways: variables were averaged by individual in each age group (to eliminate within-subject variation in age groups, and examine only between-individual variation), or scaled and centred within subjects by age group (to eliminate between-individual variation and examine only within-individual variation; van de Pol and Wright, 2009). Relationships between averaged variables (which produced one data point per

individual per age class) were assessed using pairwise Pearson product-moment correlations, separately for each age group. In addition, relationships between scaled and centred variables (which resulted in multiple data points per individual, per age class) were analyzed using repeated measures correlation r_{rm} (Bakdash and Marusch, 2018) separately for each age group. P -values for each analysis in each age group were adjusted for multiple comparisons according to Benjamini and Hochberg (1995).

3. Results

3.1. Age-related changes

Vocalisations emitted during pre-weaning tests were markedly different from those emitted at 6 months of age and older. Pre-weaning age kittens showed more variability in their number of vocalisations (sometimes vocalising over 100 times during the test), had a higher F_0 and a wider range of $SD F_0$ (Fig. 3). In addition, pre-weaning kittens had a wider range of temperature change of the nose (Fig. 3).

3.2. Repeatability

Agreement repeatability and adjusted repeatability were calculated for all variables within and between age classes (Tables 3 and 4). We found that all of the behavioural variables (motor activity, number of vocalisations and at least some of the associated acoustic parameters) were repeatable within every age class. These variables were also repeatable between age classes, and both their within- and between-age class repeatability increased with age, with the exception of the standard deviation of the fundamental frequency ($SD F_0$) which was only repeatable at pre-weaning and 12 months of age (Tables 3 and 4).

With regards to the IRT data, however, there was no significant agreement nor adjusted repeatability of the change in eye temperature for any age class (Tables 3 and 4). Temperature change of the nose was moderately repeatable within the age classes at 2 and 6 months for agreement and adjusted repeatability (Tables 3 and 4) as well as at 12 months of age for adjusted repeatability (Table 4), but for neither form of repeatabilities at 18 months of age. Temperature change of the nose showed moderate repeatability between the ages of pre-weaning–6 months, and 6–12 months, as well as across all age classes combined (Table 5).

There was little difference between agreement repeatabilities (including only individual identity as a random effect) and adjusted repeatabilities (including additional fixed effects of trial, sex, age, age*sex, or maternal identity as a random effect; Tables 3 and 4). The small differences between the two types of calculations indicate that

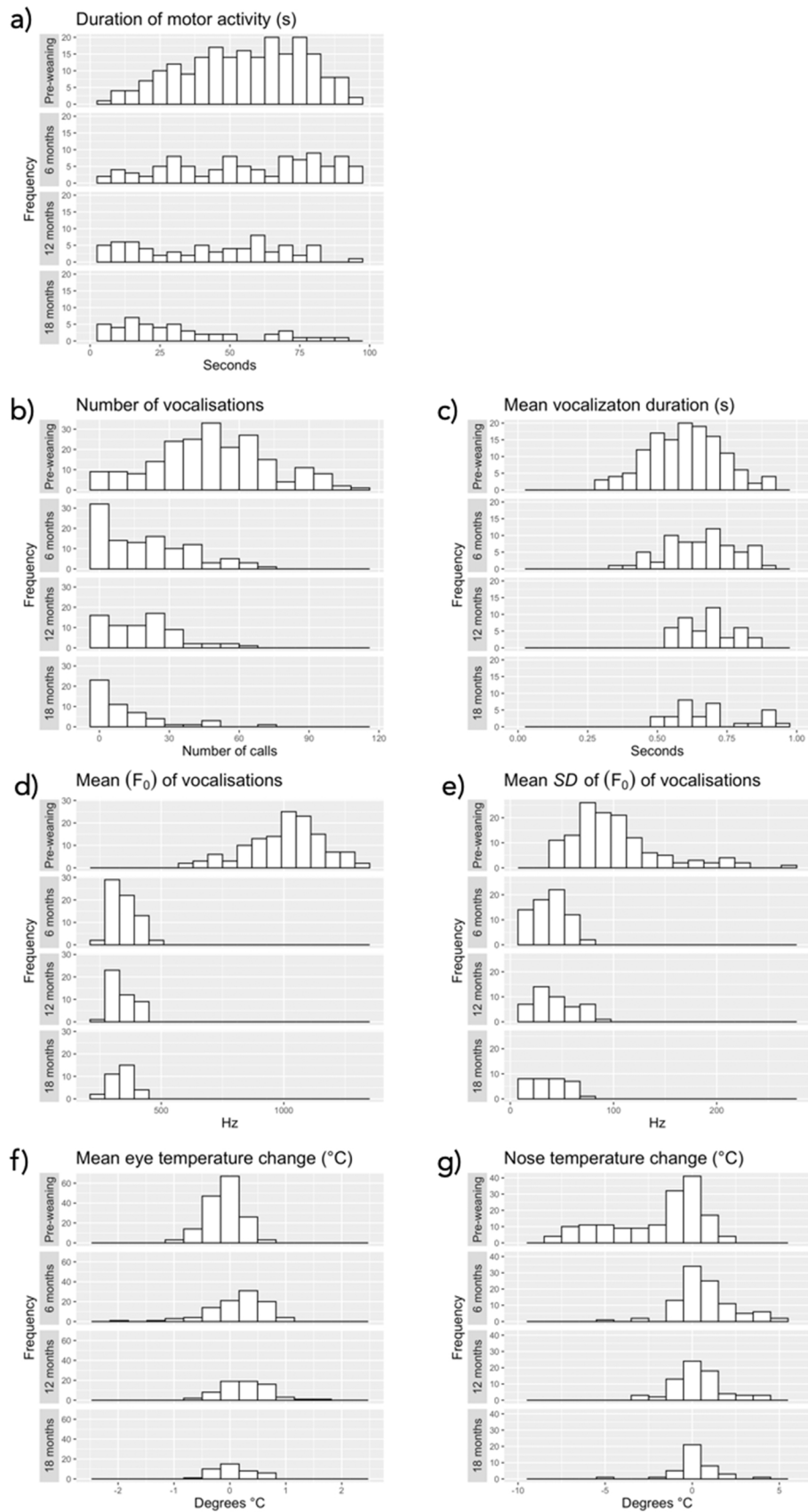


Fig. 3. Histograms of the responses measured in the separation/confinement tests in each age group. All trials from each age group are included. a) Duration of motor activity, b) Number of vocalisations emitted in a trial, c) Mean duration (s) of the vocalisations in a trial, d) Mean fundamental frequency (F_0) of the vocalisations in a trial, e) Mean standard deviation of the fundamental frequency ($SD F_0$) of the vocalisations in a trial, f) Mean temperature change of the eyes after the trial ($^{\circ}C$), g) Mean temperature change of the nose after the trial ($^{\circ}C$).

Table 3

Agreement repeatabilities (*R*) and 95% confidence interval [95% CI] of the measured variables. Bolded values are significant ($P < 0.05$ and the 95% CI does not include 0). No covariates were included in these models.

Outcome variable	Pre-weaning	6 months	12 months	18 months
Duration of motor activity	0.245 [0.086, 0.38]	0.489 [0.271, 0.644]	0.554 [0.279, 0.73]	0.684 [0.401, 0.831]
Number of vocalisations	0.582 [0.451, 0.695]	0.724 [0.57, 0.827]	0.663 [0.412, 0.807]	0.926 [0.835, 0.966]
Mean duration of vocalisations	0.472 [0.275, 0.636]	0.558 [0.272, 0.753]	0.58 [0.211, 0.786]	0.779 [0.433, 0.912]
Mean F_0 of vocalisations	0.664 [0.493, 0.78]	0.408 [0.07, 0.672]	0.754 [0.485, 0.897]	0.553 [0.09, 0.816]
Mean $SD F_0$ of vocalisations	0.445 [0.227, 0.611]	0.329 [0, 0.611]	0.797 [0.555, 0.904]	0.477 [0, 0.777]
Temperature change in eyes (mean)	0.008 [0, 0.132]	0.175 [0, 0.406]	0.086 [0, 0.366]	0 [0, 0.396]
Temperature change of nose	0.25 [0.08, 0.418]	0.361 [0.125, 0.562]	0.242 [0, 0.486]	0.159 [0, 0.469]

(Urrutia et al.)

repeatability was not due to differences between groups (e.g. between sexes) which could inflate repeatability estimates, but rather that there were genuine individual-level differences which persisted even when controlling for potentially confounding effects.

3.3. Associations between variables

At the within-individual level, there were significant associations between several variables at pre-weaning (Table 6a): mean call duration was positively correlated with number of vocalisations ($r_{tm} = 0.433$, $P = 0.0009$), and negatively correlated with mean F_0 ($r_{tm} = -0.411$, $P = 0.002$); mean F_0 was positively correlated with $SD F_0$ ($r_{tm} = 0.375$, $P = 0.003$) and with duration of motor activity F_0 ($r_{tm} = 0.393$, $P = 0.002$); and duration of motor activity was slightly positively correlated with temperature change in the nose ($r_{tm} = 0.252$, $P = 0.03$). None of these correlations was found in later age groups. At 6 months of age, there was a negative association between the temperature change in

the nose and the number of calls ($r_{tm} = -0.448$, $P = 0.005$).

At the between-individual level, only two significant associations between variables were found (Table 6b). At 12 months of age, there was a positive association between mean F_0 and mean call duration ($r = 0.781$, $P = 0.045$); at 18 months, there was a positive association between number of vocalisations and duration of motor activity ($r = 0.59$, $P = 0.029$).

4. Discussion

4.1. Consistent individual differences across development

Our first aim in this study was to investigate the long-term stability of individual differences in response to an everyday stressor across development in the domestic cat. We found robust evidence for this in the two behavioural measures used, motor activity and vocalisation. There were stable individual differences across the three trials conducted within and

Table 5

Between-age agreement repeatabilities (*R*) and 95% confidence interval [95% CI] of the measured variables. Bolded values are significant ($P < 0.05$ and the 95% CI does not include 0). No covariates were included in these models. Note that temperature change in the eyes was excluded as it was not repeatable within any age group.

Outcome variable	Pre-weaning–6 months	6–12 months	12–18 months	All ages
Duration of motor activity	0.27 [0.153, 0.382]	0.413 [0.248, 0.554]	0.533 [0.32, 0.682]	0.263 [0.16, 0.365]
Number of vocalisations	0.352 [0.226, 0.466]	0.649 [0.503, 0.755]	0.684 [0.486, 0.795]	0.414 [0.298, 0.519]
Mean duration of vocalisations	0.447 [0.289, 0.589]	0.553 [0.342, 0.706]	0.659 [0.408, 0.8]	0.398 [0.254, 0.525]
Mean F_0 of vocalisations	0.192 [0.038, 0.339]	0.536 [0.291, 0.693]	0.541 [0.253, 0.718]	0.434 [0.286, 0.557]
Mean $SD F_0$ of vocalisations	0.119 [0, 0.28]	0.413 [0.177, 0.599]	0.644 [0.412, 0.793]	0.297 [0.15, 0.431]
Temperature change of nose	0.213 [0.084, 0.339]	0.261 [0.095, 0.42]	0.187 [0, 0.373]	0.278 [0.166, 0.396]

Table 4

Adjusted repeatabilities (*R*) and 95% confidence interval [95% CI] of the measured variables†. Bolded values are significant ($P < 0.05$ and the 95% CI does not include 0). The covariates listed were included in the models; note that where age or age*sex are listed as covariates, they were included only in the model that included all age classes and excluded from the models that included data from single age classes †.

Outcome variable	Covariates	Pre-weaning	6 months	12 months	18 months
Duration of motor activity	(1 Mother) + age + trial	0.306 [0.159, 0.462]	0.29 [0.109, 0.527]	0.506 [0.206, 0.759]	0.681 [0.234, 0.831]
Number of vocalisations	Age + sex + age*sex + trial	0.595 [0.469, 0.709]	0.74 [0.589, 0.84]	0.679 [0.466, 0.837]	0.939 [0.872, 0.973]
Mean duration of vocalisations	Age + trial	0.487 [0.307, 0.649]	0.578 [0.307, 0.777]	0.659 [0.381, 0.854]	0.787 [0.464, 0.918]
Mean F_0 of vocalisations	Age	0.664 [0.493, 0.78]	0.408 [0.07, 0.672]	0.754 [0.485, 0.897]	0.553 [0.07, 0.809]
Mean $SD F_0$ of vocalisations	Age + trial	0.331 [0.11, 0.524]	0.301 [0, 0.593]	0.786 [0.578, 0.91]	0.454 [0, 0.762]
Temperature change in eyes (mean)	(1 Mother) + age + baseline eye temperature	0.008 [0, 0.132]	0.222 [0, 0.443]	0.01 [0, 0.257]	0.24 [0, 0.546]
Temperature change of nose	(1 Mother) + age + baseline nose temperature	0.325 [0.157, 0.546]	0.319 [0.074, 0.529]	0.349 [0.056, 0.587]	0.258 [0, 0.555]

† Note that the adjusted repeatability values for duration of motor activity and number of vocalisations reported in Table 4 differ slightly from those previously reported for the pre-weaning age kittens of this dataset (Urrutia et al., 2022). In our previous report, some litter characteristics such as litter size were included in the repeatability analysis, but in the present study, the reduction in sample size in the older age groups limited the number of variables that could be included in the analysis. To aid comparisons between age groups, we used the same fixed effects for all ages and discarded litter effects entirely.

(Urrutia et al.)

Table 6a

Within-individual level associations between variables obtained through repeated measures correlation (r_{rm}) separately for each age group. P -values were adjusted for multiple comparisons in each age group according to Benjamini and Hochberg (1995). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Age group	Variable 1	Variable 2	r_{rm}	95% confidence interval [CI; lower bound, upper bound]	P
Pre-weaning	Mean F_0 of vocalisations	Mean SD F_0 of vocalisations	0.375	[0.159, 0.557]	0.003 **
Pre-weaning	Mean F_0 of vocalisations	Mean duration of vocalisations	-0.411	[- 0.586, - 0.2]	0.002 **
Pre-weaning	Mean F_0 of vocalisations	Duration of motor activity	0.393	[0.179, 0.572]	0.002 **
Pre-weaning	Mean duration of vocalisations	Number of vocalisations	0.433	[0.233, 0.598]	0.0009 ***
Pre-weaning	Temperature change of nose	Duration of motor activity	0.252	[0.06, 0.427]	0.03 *
6 months	Temperature change of nose	Number of vocalisations	-0.448	[- 0.639, - 0.205]	0.005 **

Table 6b

Among-individual level associations between variables obtained through pairwise Pearson product-moment correlations (r), separately for each age group separately for each age group. P -values were adjusted for multiple comparisons in each age group according to Benjamini and Hochberg (1995). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Age group	Variable 1	Variable 2	r	95% confidence interval [CI; lower bound, upper bound]	P
12 months	Mean F_0 of vocalisations	Mean duration of vocalisations	0.781	[0.34, 0.94]	0.045 *
18 months	Number of vocalisations	Duration of motor activity	0.59	[0.212, 0.814]	0.029 *

(Urrutia et al.)

across the four age classes tested, which spanned three life stages (pre-weaning, juvenile, adult). Nevertheless, and perhaps unsurprisingly, the repeatability of individual differences was greater within than across age classes. Vocalisation rate during stressful situations has been found to be repeatable in the short-term in kittens and young mice (Hudson et al., 2015, 2017; Urrutia et al., 2022), in adult cats (Urrutia et al., 2019; Martínez-Byer et al., 2020), as well as in other mammals such as young horses (Pérez Manrique et al., 2019, 2021) and piglets (Špinková et al., 2018). In previous studies on cats and horses, the repeatability of individual differences in vocalisations was greater than for motor activity, consistent with our findings in the present study. Given the considerable number of repeated trials (up to 12 per individual) and the consistency of the findings with previous reports, these results seem to be reliable.

Such repeatability was the more remarkable given the extensive developmental changes that cats undergo from pre-weaning age into adult life, including continued growth (Opsomer et al., 2022), physiological changes (e.g. Chugani et al., 1991), further development of behaviours involved in hunting (Adamec et al., 1980) and play (reviewed by Delgado and Hecht, 2019). In addition, the cats underwent abrupt changes in lifestyle when they moved to a diverse range of household environments at adoption (after their first set of behavioural tests). For example, whereas some were exclusively indoor cats, others became indoor-outdoor cats; whereas some entered single-owner households, others became members of households with more than one human, sometimes with children, sometimes with other cats and/or dogs; with different schedules of feeding, cleaning and other forms of care; and with different amounts of experience with pet carriers.

How could such individual differences arise at such an early age, and among littermates reared under standardized conditions? Certainly, genetic factors may have played a role, although in our dataset the (possibly multiple) paternity of the litters was not known (Say et al., 1999). One approach to investigating this could be to test for differences on the present measures between genetically more homogeneous pure breeds of cats (e.g. Marchei et al., 2009) and in kittens from litters of known paternity (McCune, 1995). However, it should be noted that stable individual differences in behaviour have been found even in genetically identical (clonal) individuals of several species, (e.g. pea aphid, *Acyrtosiphon pisum*: Schuett et al., 2011; mourning gecko, *Lepidodactylus lugubris*: Sakai, 2018; domestic pig: Archer et al., 2003), and even genetically identical individuals reared in closely controlled, homogenous environments show individual differences (Bierbach et al., 2017). Even small individual differences in the early developmental environment can create feedback mechanisms which place individuals

on different developmental trajectories and thereby lead to and reinforce consistent individual differences in behaviour, including in how they respond to, or “cope” with stressful situations (Stamps and Groothuis, 2010; Lewejohann et al., 2011; Bierbach et al., 2017). Although in the present study we cannot distinguish between the effects of genetics and early environment, the individual differences in behaviour observed in kittens persisted throughout development and into adulthood.

But what do such behavioural differences mean, how should they be interpreted, and what is their physiological and motivational basis? A first attempt to gain some insight into such issues was the reason for our second aim: to investigate possible associations between individual differences among the variables tested, which could aid our ability to identify and interpret individual differences in responding to a stressful situation, and perhaps more broadly, to identify individual differences in coping style.

4.2. Lack of association between behaviours

We found little evidence for stable associations among the behavioural measures analyzed, sometimes referred to as behavioural syndromes (Sih et al., 2004). The general lack of association between measures of motor activity and vocalisation at all ages except for 18 months is consistent with our previous studies in kittens, mice, adult cats and young horses (Hudson et al., 2015, 2017; Urrutia et al., 2019; Pérez Manrique et al., 2019, respectively). As we have previously discussed, this apparent disjunction possibly points to the existence of distinct emotional or functional domains rather than to correlated groups of behaviours (behavioural syndromes) with common underlying developmental and evolutionary origins, and common underlying mechanisms.

At pre-weaning age, there were several associations between variables at the within-individual level only. These were mainly based around vocalisation (Table 6a) and were not seen at later ages. At pre-weaning age, there were within-individual level correlations between the number of vocalisations and mean call duration, and between mean F_0 , mean SD F_0 and mean call duration, which disappeared at later ages. In domestic kittens, the vocal repertoire is not yet fully developed (Moelk, 1944). In addition, the pre-weaning age kittens of this study lived with their mother and siblings in a single room of the house; the separation/isolation test was the first time they experienced being alone in an unfamiliar space. Thus, it is likely that the vocalisations they emitted during the test were of a single type: separation calls, which provide a strong stimulus for the mother to retrieve them (Bánszegi et al., 2017). A single call type expressed in this age group could explain

the within-individual correlations observed between the vocal parameters. At pre-weaning age, there was also a small positive correlation between the temperature change of the nose and the duration of motor activity (Table 6a). However, its low r_{tm} value and the 95% CI which included a near-zero value indicate that this may not be a biologically meaningful association; in addition, it was absent in other age groups.

At 6 months of age, there was a negative correlation at the within-individual level between the temperature change of the nose and the number of vocalisations (Table 6a). It is unclear whether decreased nose temperature in this case was due to a change in breathing rate associated with increased vocal activity (Travain and Valsecchi, 2021), due to breathing or sniffing not necessarily related to stress but perhaps rather to “curiosity” and to inspecting the environment, or because higher vocalisation rate and temperature change of the nose are both indicative of a response to acute stress (Nakayama et al., 2005). However, because this correlation was present only in one age group, and temperature change of the nose was only moderately repeatable (Tables 3 and 4), we believe that this correlation is not strongly indicative of an emerging association between these measures.

At 18 months of age, there was a positive correlation at the among-individual level between number of vocalisations and motor activity (Table 6b). This correlation was present only in this age group, and it is important to note that the 95% CI of this correlation is quite wide. At 18 months of age, the cats did not vocalise in approximately half of the trials (Fig. 3), which in conjunction with the reduced sample size at this age could contribute to this wide range. Whether this correlation points to an emerging behavioural syndrome or is a side-effect of age-related changes in vocalization rate later in life requires further investigation.

4.3. Vocalisation

It has been previously reported that vocalisations emitted by domestic cats during confinement in a carrier have lower F_0 and SD of F_0 than those emitted in other, positive situations (Schötz et al., 2019), and that kitten isolation calls emitted during situations of high negative arousal have lower F_0 than those emitted during low arousal (Scheumann et al., 2012). Our results now indicate that stable individual differences in number of calls, mean F_0 , mean SD of F_0 , and mean call duration exist within a single context and are consistent across the long term. These repeatable individual differences persisted despite general age-related changes in the number of vocalisations emitted and their characteristics (Fig. 3). Thus, a more detailed investigation of these parameters should be useful to gain a better understanding of the emotional meaning of vocalisations and their development, and particularly given their potential usefulness in the investigation of individual differences in behaviour and the assessment of animals' emotional state in everyday contexts (Schrader and Todt, 1998; Briefer, 2012; Pérez Manrique et al., 2021).

4.4. Thermography

We found moderate repeatability (agreement and adjusted) of the change in nose temperature at all ages except for 18 months, as well as moderate agreement repeatability of this variable across development (Tables 3–5). During stress, sympathetically mediated vasoconstriction should theoretically cause nose temperature to drop (Kano et al., 2016; Schraft and Clark, 2017; Dezecache et al., 2017), however we observed a mix of increasing and decreasing temperatures at all ages, and in fact, temperature increase was more common than decrease in trials at 6, 12 and 18 months (Fig. 3). Perhaps a temperature drop caused by stress-mediated vasoconstriction was masked by other factors also affecting nose temperature, such as sniffing, vocalisation, altered breathing rate, or rubbing against the carrier, which in turn may or may not be indirectly caused by stress (Travain and Valsecchi, 2021). On the other hand, in the majority of trials of pre-weaning age kittens, nose temperature decreased as expected. One possibility is that this was due

to loss of body heat, since the kittens were tested at 5, 6 and 7 weeks of age and thermoregulation is only fully developed at 7 weeks of age (Olmstead et al., 1979). If this were the case, we would expect to see a positive association between change in nose temperature and age (in weeks) and/or body mass in the pre-weaning age kittens. However, we found no such meaningful associations (results not shown), suggesting that the decrease in nose temperature in this age group was not due to inability to properly thermoregulate. This leaves several other –not mutually exclusive– possibilities: any conflicting processes which may have masked decreases in nose temperature in older cats could be reduced or absent in pre-weaning aged kittens; there may be other processes present in kittens, but not older cats, which result in decreased nose temperature; stress-induced peripheral vasoconstriction may have a more pronounced effect in kittens; or perhaps the pre-weaning age kittens were more stressed by the test, since at this age it involved separation from the litter. It is still important to note, however, that although nose temperature decreased in most pre-weaning age trials, it increased in 25% of these trials, so even in this age group we cannot conclude that change in nose temperature is a clear indicator of stress. Finally, although modestly repeatable at most ages, the change in nose temperature showed no clear nor consistent association with motor activity nor vocalization. Therefore, we cannot conclude that the moderately repeatable changes in nose temperature were caused –or were purely caused– by a response to acute stress.

It has been previously reported that exposure to a stressful situation is accompanied by changes in core body temperature in domestic kittens, although this change could be positive or negative (Marchei et al., 2009) and its repeatability is unknown. The temperature of the medial canthus of the eye is reportedly correlated with core body temperature during rest and activity (Zanghi, 2016), and in domestic cats the average eye temperature has also been correlated with scores on the Feline Temperament Profile questionnaire, which categorises individuals based on their reactions towards humans (Foster and Ijichi, 2017). However, our results indicate that changes in eye temperature in response to an acute stressor are not repeatable in domestic cats, and therefore this region may not be adequate for the assessment of acute stress in this species. Other regions of the body, such as the tympanic membrane, have been previously associated with serum cortisol levels after a stressful situation in the domestic cat by Mazzotti and Boere (2009). However in that study single measures were used, and individual differences in baseline temperature and serum cortisol concentration were not evaluated. The temperature change of the nose, eye, tympanic membrane, or other regions in response to acute stress still require further validation before they can be used as reliable indicators of an acute stress response. Therefore, further research using repeated measures and additional physiological measures is required.

4.5. Limitations

As described in the Methods section, for the 6-, 12- and 18-month old cats, the separation/confinement test was performed following a series of three other short behavioural tests. These involved brief handling, provision of a small piece of palatable food, and brief exposure to a live mouse in a jar. To experimentally control for the potential carryover effects on superficial temperature caused by the previous tests, the same order of tests was maintained (our sample size would have been insufficient to randomize test order and control for it statistically). In addition, to minimize any such carryover effect, there was a 5–10-minute break from testing before the start of the separation/confinement test; this length of time has been found sufficient for eye temperature to return to baseline in domestic dogs after manipulation (Travain et al., 2015) and exposure to palatable food (Travain et al., 2016), and in mice after exposure to predator odor (Lecorps et al., 2019). In addition, for the repeatability analysis we used the repeatability of the change in temperature with respect to baseline, rather than post-test temperature itself. Finally, we added baseline temperature as a fixed effect to the

adjusted repeatability analysis (Table 4), which would control for carryover effects from previous tests as well as individual variation in ambient temperature, humidity, etc. between individual trials (Jerem et al., 2019).

A final limitation of the present study, common to longitudinal studies, is the reduction in sample size that occurred between pre-weaning and 18 months of age (Table 1). This was mainly due to difficulties in test scheduling (e.g. unresponsive owners or conflicting schedules), families moving away from the city, or technical issues leading to post-test data loss; only one missing and two dead cats were reported during the study. Therefore, we consider that the causes of reduction in sample size would not have introduced a selection bias into the dataset; in addition, confidence intervals are reported for all results to aid in their interpretation.

4.6. Applied relevance

In the present study, IRT revealed that the change in nose temperature was more stable than the change in eye temperature, although its repeatability within ages and across development was low. Such consistently low repeatability indicates the importance of basing thermographic measurements of animals on multiple, rather than on just one or few, trials. Furthermore, the thermographic measures were largely unrelated to the behavioural measures and, perhaps most significantly, to vocal measures as established indicators of emotional state, including stress (Briefer, 2012). Taking these points together, we suggest that the usefulness of facial thermography as an indicator of acute stress in applied contexts, such as assessing the welfare of companion or production animals, is presently limited.

Nevertheless, the brief confinement/separation test and simple behavioural measures used in the present study might be useful in phenotyping individuals to help match them to appropriate new homes. For example, a more reactive, possibly stress-prone cat might be better suited to the quieter environment of an older single owner than to the rough and tumble of a multi-person, multi-cat/dog household (Foster and Ijichi, 2017; Martínez-Byer et al., 2020). In this regard, simply counting the number of vocalisations emitted during a given time in response to a brief stressor such as confinement in a carrier would seem to be a particularly useful measure as it is cheap and easy to implement. However, whether it is a good indicator of the amount of stress subjectively experienced by an individual still needs to be validated by physiological indicators of stress conducted in parallel (Rushen, 2000; cf. Pérez Manrique et al., 2021, in horses). This was our intention in the present study with the use of facial thermography which, however, as discussed above, does not seem presently useful for this purpose in the cat.

5. Conclusions

Mixed-breed cats of both sexes show stable individual differences in the duration of motor activity and in the number, duration and pitch of vocalisations in response to an everyday, acute stressor when repeatedly tested across development. The developmental origins and mechanisms underlying such robust and stable individual differences in behaviour remain unclear and require further investigation.

Individual differences in performance on the two main behavioural variables of the present study (motor activity and vocalisations) were unrelated to each other, although there were some associations between different vocal parameters which suggest that even a simple measure such as number of vocalisations may potentially reflect the degree of acute stress.

Although IRT has been proposed as a convenient means of assessing emotional and physiological responses to acute stressors in animals, the results obtained in the present study do not support its application as a reliable indicator of acute stress in the domestic cat.

The cat is particularly suitable for the investigation of the

development of individual differences in behaviour in a mammal given its ready availability and ease of observation and testing outside the laboratory, and thus for continuing efforts to develop reliable indicators of individual differences in the degree of stress animals may experience in everyday contexts.

Ethical note

Throughout the study, animals were kept and treated according to the guidelines of International Society for Applied Ethology (Sherwin et al., 2003), as well as the relevant legislation for Mexico (National Guide for the Production, Care and Use of Laboratory Animals, Norma Oficial Mexicana NOM-062–200–1999).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability statement

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

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Discusión General

Esta tesis examina el desarrollo de las diferencias individuales estables en el comportamiento del gato doméstico. Usamos un diseño longitudinal para evaluar a un grupo de individuos a los 2, 6, 12 y 18 meses de edad en cuatro pruebas conductuales biológicamente relevantes. Encontramos diferencias individuales estables (repetibles) a corto plazo dentro de todas las clases de edad incluidas en el estudio; y en tres de las cuatro pruebas realizadas, el valor de repetibilidad R de las conductas evaluadas tendió a aumentar con la edad. Este aumento relacionado a la edad se debió tanto a la reducción de la varianza intra-individual como al aumento de la varianza entre individuos.

Adicionalmente, la repetibilidad a largo plazo (que abarca todos los grupos de edad) tendió a ser baja o, como mucho, moderada. Sin embargo, entre grupos de edad consecutivos (es decir, de 2 a 6, 6 a 12 y 12 a 18 meses) la repetibilidad era más alta, sobre todo entre grupos de edad posteriores. Esto sugiere que aunque a muy largo plazo la repetibilidad de las conductas fue relativamente baja, esto no se debió a alguna reorganización repentina de la conducta en algún momento particular del desarrollo, sino a que los comportamientos de los individuos cambiaron gradualmente a lo largo de un período de tiempo más largo.

No encontramos evidencia de síndromes conductuales en ningún grupo de edad, a diferencia de los resultados obtenidos anteriormente en un estudio similar realizado en gatos adultos alojados en un refugio, donde observamos un síndrome conductual aparentemente basado en diferencias en los comportamientos orientados hacia los humanos (Anexo 1). Sugerimos que la discrepancia entre los resultados en estos dos grupos de gatos puede deberse a que los individuos que participaron en el presente estudio longitudinal tenían antecedentes relativamente homogéneos y experiencias en general positivas con humanos, en contraste con el origen heterogéneo y la actitud mixta hacia los humanos de los gatos de refugio estudiados previamente (Anexo 1). La

presencia de comportamientos repetibles y la estructura de los síndromes conductuales pueden variar entre poblaciones que están expuestas a diferentes condiciones (p. ej., Dingemanse et al., 2007; Evans et al., 2010; Michelangeli et al., 2019); ésta es un área de estudio interesante en la que el gato doméstico puede ser un buen modelo de estudio, ya que existen a nivel mundial como animales de compañía, callejeros (con contacto limitado con humanos), o ferales. También cabe notar que en los tres estilos de vida, los gatos tienen un gran impacto en los ecosistemas no solo a través de su depredación directa de especies nativas, sino también a través de otros mecanismos como la transmisión de enfermedades y el cambio de comportamiento en las especies presa (Medina et al., 2014). Investigar la estructura de la personalidad de los gatos domésticos en diferentes condiciones podría no sólo elucidar cómo la estructura de los síndromes conductuales varía entre poblaciones, pero además puede conducir a una mejor comprensión de sus diversos impactos en la vida silvestre.

Observamos diferencias individuales estables (repetibles) en varias medidas acústicas de las vocalizaciones emitidas en respuesta a una breve prueba estresante: aislamiento en una jaula transportadora. Estas diferencias individuales estaban presentes dentro y entre las clases de edad, lo que sugiere que la vocalización puede ser un buen indicador de la respuesta al estrés/estilos de afrontamiento en el gato. La repetibilidad de la tasa de vocalización en respuesta al estrés se ha reportado en otras especies (por ejemplo, carbonero de capucha negra, *Poecile atricapillus*: Guillette & Sturdy, 2011; cerdo doméstico, *Sus scrofa domestica*: Friel et al., 2016; Špinko et al., 2018), aunque muy pocos estudios han examinado su repetibilidad a largo plazo (pero véase Manrique et al., 2021). Los mecanismos que subyacen a la respuesta fisiológica al estrés también pueden controlar o contribuir a las diferencias individuales consistentes en la actividad vocal en respuesta al estrés a lo largo del desarrollo. Por ejemplo, Brunelli y Hofer (2007) seleccionaron artificialmente dos linajes de ratas basándose en su tasa de vocalización ultrasónica (alta o baja)

en respuesta al aislamiento cuando eran crías; en etapas de edad posteriores, los dos linajes se diferenciaron en su respuesta cardíaca a una situación estresante, mediada por el sistema nervioso simpático, lo que sugiere que estas dos respuestas están vinculadas a nivel mecánico. Investigaciones adicionales sobre la relación entre la respuesta fisiológica al estrés y la vocalización podrían elucidar la relación entre los dos, incluyendo a nivel mecanístico. Esto podría realizarse en conjunto con análisis más detallados de las vocalizaciones, por ejemplo examinando cómo cambian las características de las vocalizaciones a lo largo de una prueba o situación estresante, examinando el contorno de la F_0 , etc.

Finalmente, el cambio en la temperatura superficial del ojo después de una breve prueba estresante no mostró patrones consistentes de cambio a ninguna edad, ni relación alguna con la vocalización, a pesar de que en otras especies se ha reportado un aumento en la temperatura del ojo en respuesta al estrés agudo (revisado por Travain y Valsecchi, 2021). Aunque en otras especies la temperatura del canto interno del ojo se correlaciona con la temperatura corporal central durante el reposo y la actividad (Zanghi, 2016), y en gatos la temperatura corporal central se ha relacionado con diferencias individuales en la respuesta ante humanos evaluada por medio de cuestionarios (Foster y Ijichi, 2017), puede que esto no se traduzca a cambios en la temperatura superficial del ojo en respuesta a estrés agudo. Por otro lado, encontramos diferencias individuales moderadamente repetibles en algunos grupos de edad en el cambio de la temperatura superficial de la nariz. Sin embargo, éste no se correlacionó de manera consistente con la conducta ni con aspectos de vocalización, por lo que no es claro que pueda considerarse como indicador de la respuesta al estrés agudo. Sugerimos que es necesario validar esta y otras medidas de temperatura superficial con algunos indicadores fisiológicas adicionales de estrés (p. ej., concentración de cortisol en plasma) antes de utilizarlas como indicador de la respuesta al estrés en contextos aplicados.

A pesar de la gran cantidad de estudios de personalidad realizados en animales adultos, todavía hay relativamente pocos estudios que se centren en el desarrollo de la misma (Cabrera et al., 2021). En los capítulos anteriores mostramos que las diferencias individuales en el comportamiento del gato doméstico están presentes desde una edad muy temprana y se vuelven cada vez más repetibles a lo largo del desarrollo y en la vida adulta, debido a cambios en la varianza intra- e inter-individual. Además, no observamos síndromes conductuales, a diferencia de los resultados anteriores en una población diferente de gatos. Quedan por explorar los mecanismos que conducen al aumento de la repetibilidad y cambios en la varianza de la conducta con la edad, y las diferencias en la estructura de la personalidad entre las poblaciones de esta especie.

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Anexo I


Evidencia de diferencias individuales en la conducta y de síndromes conductuales en
gatos domésticos adultos

Evidence for individual differences in behaviour and for behavioural syndromes in adult shelter
cats

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Article

Evidence for Individual Differences in Behaviour and for Behavioural Syndromes in Adult Shelter Cats

Sandra Martínez-Byer ¹, Andrea Urrutia ^{1,2}, Péter Szenczi ^{3,*}, Robyn Hudson ² and Oxána Bánszegi ^{2,*} 

¹ Posgrado en Ciencias Biológicas, Unidad de Posgrado, Edificio A, 1er Piso, Circuito de Posgrados, Ciudad Universitaria, Coyoacán, CP 04510, Mexico; brownie_byer@ciencias.unam.mx (S.M.-B.); andreaurrutia@outlook.com (A.U.)

² Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, A.P. 70228, CP 04510, Mexico; rhudson@biomedicas.unam.mx

³ CONACYT—Instituto Nacional de Psiquiatría Ramón de la Fuente Muñiz, Unidad Psicopatología y Desarrollo, Calz. México-Xochimilco 101, CP 14370, Mexico

* Correspondence: peter.szenczi@gmail.com (P.S.); oxana.banszegi@gmail.com (O.B.); Tel.: + 52-56-22-38-28 (P.S.); + 52-56-22-38-28 (O.B.)

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Simple Summary: An important activity of modern animal shelters is the development of successful adoption programmes. In this regard, there is a need for reliable tests of individual differences in behaviour to help match the “personality” of potential adoptees with the lifestyle and needs of prospective owners; a companion animal for an elderly person remaining at home requires a different match than a pet for someone who will be away most of the day; a pet kept exclusively indoors in a small apartment requires a different match than an indoor/outdoor pet. In the present study, we repeatedly tested 31 mixed-breed adult cats of both sexes and a wide range of ages in five behavioural tests at a shelter in Mexico City, Mexico. The tests were designed to be easily implemented by shelter staff, and were short and low cost and intended to simulate common situations in a pet cat’s everyday life. We found consistent (stable) individual differences in the cats’ behaviour on all five tests, as well as correlations between their behaviour across tests. This suggests that such tests may contribute to reliably characterizing the “personality” of individual cats and so help increase the rate of successful adoptions.

Abstract: Consistent inter-individual differences in behaviour have been previously reported in adult shelter cats. In this study, we aimed to assess whether repeatable individual differences in behaviours exhibited by shelter cats in different situations were interrelated, forming behavioural syndromes. We tested 31 adult cats in five different behavioural tests, repeated three times each: a struggle test where an experimenter restrained the cat, a separation/confinement test where the cat spent 2 min in a pet carrier, a mouse test where the cat was presented with a live mouse in a jar, and two tests where the cat reacted to an unfamiliar human who remained either passive or actively approached the cat. Individual differences in behaviour were consistent (repeatable) across repeated trials for each of the tests. We also found associations between some of the behaviours shown in the different tests, several of which appeared to be due to differences in human-oriented behaviours. This study is the first to assess the presence of behavioural syndromes using repeated behavioural tests in different situations common in the daily life of a cat, and which may prove useful in improving the match between prospective owner and cat in shelter adoption programmes.

Keywords: individual differences; behavioural assays; behavioural syndromes; companion animal; *Felis silvestris catus*; shelter cats; human-cat relation

1. Introduction

For years, the domestic cat (*Felis silvestris catus*) has been among the most popular pets in the world [1,2]. Interest in cat behaviour, and particularly in inter-individual differences (animal personality), is reflected in recent reviews [3–6] and special issues in scientific journals treating such topics [7,8]. The cat is a good candidate for the study of individual differences as it is readily accessible and has a rich behavioural repertoire. It is also by far the most studied feline species in this respect [3]. As with other domestic animals (companion, farm and working animals), taking into account cats' personality differences when rehoming or selecting them for specific tasks can have implications for management, welfare and economy [3,9,10].

Broadly defined, animal personality refers to relatively stable inter-individual differences in behaviour [11–13]. When several of these behaviours correlate across contexts, they can be characterized as a behavioural syndrome [12,14,15]. The most common methods used to study individual differences in behaviour in the cat include observation [16,17], owner surveys [18,19] and behavioural tests [20,21]. The latter have the advantage that they can be used to evaluate and quantify the stability of individual differences across repeated standardised testing. Since an individual's behaviour is expected to be variable to some degree, some behaviours may be inconsistent and therefore less informative of the individual's behaviour at a later time. Therefore, when testing cats, reliable methods are needed, i.e., behavioural tests and measures that have been found to be highly repeatable.

Many studies of cat personality or temperament are based on behavioural observation ([3,4] see reviews), which provide important information about cats' behaviours in their daily environments. However, to explore cats' reactions to specific situations, behavioural tests are necessary. The two most commonly used tests in cat personality research are novel object tests, where the animal is presented with an unfamiliar object, and tests of reaction to either familiar or unfamiliar humans [3]. Novel object tests tend to use stimuli of unclear biological relevance (e.g., a fan with paper streamers, a remote-control car, a metal container with a spring, or a wooden box; [20,22,23]). While these tests have been reported to reveal individual differences, their meaning in daily situations of the life of the cat is unclear. Therefore, in the present study, we decided to test the behavioural responses of cats to situations corresponding to what they would likely encounter in real-life situations.

Given cats' popularity as companion animals, there has been a tendency to study their individuality in terms of their interaction with humans, for example, in their reaction to approach or handling by a familiar or unfamiliar person [20,24–27]. Other behaviours of interest for both companion and working cats (particularly mousers) include their reaction to everyday stressful situations or to prey, respectively. However, we are unaware of any studies that have experimentally addressed the inter-individual consistency of behavioural differences in these situations. Nevertheless, animal shelters have begun to implement personality testing as part of their adoption programmes, favouring a combination of surveys and behavioural testing, as in the Feline Temperament Profile [21] and the Meet Your Match Feline-ality assessment [28].

The present study is the first to incorporate repeated measurements using several behavioural tests and to take a behavioural syndrome approach by evaluating correlations among these measurements in a heterogeneous population of cats (wide age range, different backgrounds) housed in an animal shelter. Animal shelters have a continuing need for reliable personality tests, for example, to better match potential pets with prospective owners and households or to identify cats that may better fit a specific situation, such as working or therapy cats. We used five behavioural assays that we consider to be ethologically and ecologically relevant to the daily life of the domestic cat, repeated three times each (see details below). We previously reported an analysis of data which included a subset of the data presented in the present paper, gathered during the separation/confinement test [29], but here we include further behavioural tests with the aim of identifying a larger range of repeatable individual differences and behavioural syndromes.

2. Materials and Methods

2.1. Study Site and Animals

We collected data from 31 adult cats (14 males and 17 females) from a shelter in Mexico City, Mexico, aged between 1 and 11 years (mean 4.5, SD 2.6, Supplementary Material Table S1). In some cases, the cats' ages were not known with certainty and were estimated by veterinarians. Participants were chosen randomly from among the cats at the shelter, which were in good health and permitted handling. All the cats had been neutered and had received post-operative care by qualified veterinarians within three days of entering the shelter, and all cats participating in the study had been at the shelter for at least six weeks prior to the start of behavioural testing. The shelter was a four-storey house divided into sections; approximately 50 cats were housed in each section according to how well they tolerated each other. All sections consisted of at least two rooms (approx. 2.5 × 3.5 m each) with access to a fenced outdoor area (approx. 2 × 4 m). Each cat was free to roam within its section. The rooms were furnished with cat beds, boxes of assorted sizes with blankets, scratchers and toys. Water, commercial dry cat food and sand boxes were always available.

2.2. Procedures

Tests were performed weekly for 12 sequential weeks; each of the five tests was performed three times across three sequential weeks (the human approach tests were performed on the same days). One test was performed per day on all subjects, tested in randomized order between 13:00 and 18:00. Not all cats were available for all trials, therefore sample sizes differ slightly between the tests (see Supplementary Material Table S1 for information on which cats participated in each test). All tests were video recorded (GoPro[®] Hero3+, GoPro, Inc., San Mateo, CA, USA) for subsequent behavioural analysis.

2.3. Behavioural Testing

2.3.1. Struggle Test

We proposed the struggle test as a proxy for the handling tests used in different mammalian [30–33] and bird species [34–36]. Since domestic cats are frequently handled by their owners, by other familiar and unfamiliar humans, and by veterinarians, we redesigned this test to evaluate the struggle response when they are picked up and restrained. We tested 30 adult cats (13 males and 17 females; mean age 4.5, SD 2.6 years, min = 1, max = 11). The test was performed in the section of the shelter where the cat normally resided. One of the experimenters (S.M.-B.) approached the cat and stroked it three times from the head to the base of the tail, then picked it up, holding it with both hands around the thorax, under its forelimbs. The test lasted until the cat began to struggle (see Table 1 for behavioural definition) or until 30 s elapsed after picking it up. When this happened, the cat was immediately set down. The experimenter wore gloves as a precaution against scratches.

Table 1. Behavioural variables recorded in each test.

Behaviour Measured	Definition
Struggle test	
Struggle (latency)	Lifting one of the hind paws and touching or kicking the experimenter's forearm
Separation/confinement test	
Vocalization (latency and number)	Meow-type vocalizations
Motor activity (latency and duration)	Displacement of any of the limbs on the floor or sides of the carrier for at least 1 s

Table 1. Cont.

Behaviour Measured	Definition
Mouse test	
Near the mouse (latency and duration)	At least the front paws within 50 cm of the jar containing the mouse
Tail swishing (duration)	Any time the cat swished its tail from side to side at least twice
Interaction (latency and duration)	Contact with the jar, either sniffing or pawing
Walking around the jar (duration)	Walking from one side of the jar to the other while near it
Passive human approach test	
Approach score (1–5)	Maximum degree of proximity to the unfamiliar human
Vocalization (latency and number)	Meow-type vocalizations
Finger–nose contact (binary)	If the cat established contact by touching its nose to the human’s outstretched finger
Active human approach test	
Stroke (latency)	Latency to the first full stroke from head to tail in a set by the unfamiliar person

2.3.2. Separation/Confinement Test

Separation/confinement tests are used for personality testing in many animals, particularly in social species [37–41]. Despite the fact that cats are considered only facultatively a social species [42,43], in previous studies this type of test has been successfully used for evaluating individual differences in kittens of the domestic cat [44,45] and adult shelter cats [29]. Moreover, this test represents a common situation in a cat’s daily life around humans, since cats are often confined in a carrier to take to other places outside their home.

The data from this test combined with other data from additional shelter cats have been previously reported in Urrutia et al. [29]. We tested 28 adult cats (12 males and 16 females; mean age 4.6, SD 2.7 years, min = 1, max = 11). Tests were performed in a small closed room unfamiliar to the cats; the room was 1.5 × 2 m, with flat-finished, unpainted concrete floor, walls and ceiling, and without furnishings. During the test, no other animals or humans were allowed to enter either the test room or the room adjacent to it to limit auditory and olfactory contact. One experimenter approached the cat (either S.M.-B. or A.U.), briefly stroked it and then carried it in her arms into the test room. With the help of a second experimenter, they placed the cat inside a standard commercial pet carrier (42 × 61 × 38 cm), which was a closed plastic box with a steel grill door at one end and ventilation holes along the sides. The carrier, with the cat inside, was then placed on the floor at a previously marked position and the experimenters left the room. The test lasted two minutes. Once this time had elapsed, the cat was removed from the carrier and returned by one of the experimenters to its home room. The video camera was set up 60 cm from the carrier. To improve visibility, a red light was mounted inside the carrier. The carrier was cleaned between trials with isopropyl alcohol. See Table 1 for definitions of the behaviours analysed in this test.

2.3.3. Mouse Test

In our experience, neither kittens nor adult cats show sustained interest in interacting with the types of inanimate objects conventionally used in novel object tests. We therefore chose tame, laboratory-strain (BALB/c) mice as the “novel object” to more closely approximate a biologically relevant stimulus, since small rodents are the most common prey of the domestic cat [46–50] and because of the ease with which they can be maintained and handled (see below for details on how the mouse was presented; see also [51]). In a previous study by Yang et al. [52], the BALB/c mouse strain was found to show the least fearful reactions in response to a predator. In our tests, a total of five mice were used in rotation; three of them were taken to the shelter on test days. The mouse in the jar was switched every two trials (approx. 10 min) to minimize stress. The stimulus animals showed no obvious signs of fear in the presence of the cats; there were no signs of panic (e.g., freezing) or attempted escape or defence (e.g., jumping), they moved around in the jar in apparent calm, sometimes adopting

the stretch–attend posture—which according to previous research is indicative of risk assessment rather than a fearful reaction [52]—in apparent curiosity at the presence of the cats. At the end of the study, the mice were adopted by student participants. For more details on the housing of the mice outside the tests, see Supplementary Material File S2. Additionally, during pilot tests, thermal pictures of the mice were taken before and after being in the jar with a cat in the room. Analysis of these images showed that the stress experienced by the mice (as measured by the increase in eye temperature) was comparable to that experienced in routine laboratory tests [53,54].

We tested 23 adult cats (7 males and 16 females; mean age 4.4, SD 2.5 years, min = 1, max = 11). Cats were individually tested in an unfamiliar room (4 × 6 m) which was cleared of all other cats and any objects that could be distracting. Subjects were given a two-minute habituation period before introducing the mouse. During habituation, and throughout the test, an experimenter (S.M.-B.) remained in the room, standing motionless and silent in a corner.

At the end of the habituation period, the experimenter restrained the cat in the middle of the room while a second experimenter brought in a mouse inside a clear, thick glass jar (15 cm in diameter × 20 cm high) with a perforated lid and covered with a cardboard box. At a marked position approximately 1.5 m from the cat and against a wall, the second experimenter fixed the jar to the floor with double-sided tape, removed the cardboard box and left the room. The first experimenter then released the cat and returned to the corner. The cat could see and presumably hear and smell the mouse but could not access it. The cat was free to interact with the jar for two minutes, after which the test ended and the cat was returned to its section of the shelter. The video camera was mounted on the wall 2 m above the jar. See Table 1 for definitions of the behaviours analysed.

2.3.4. Human Approach Tests

Human approach tests have been commonly used to evaluate cat behaviour [20,25,55–57], especially in shelters [27,58]. We modified the test from Adamec et al. [59] and tested the response of 28 adult cats (11 males and 17 females; mean age 4.6, SD 2.7 years, min = 1, max = 11) to an unfamiliar person. This person, a male volunteer, was the same person on a given test day but a different volunteer each week (age 21–25 years). To minimize unintentional odour cues, all were non-cat owners, were asked to wear fresh clothes and were unknown to the cats. Thus, the cats had the opportunity to interact with three different humans, one in each trial.

- Passive human approach test

Tests were performed in the same room as described for the mouse test. Before testing, two concentric circles, 1.5 and 3 m in diameter, were drawn on the floor with chalk to use as references of cat–human distance in the later video analysis, and the male volunteer was asked to sit cross-legged on the floor in the centre of the inner circle. When the volunteer was in position, the cat was carried in arms into the room by a familiar experimenter and placed in a shallow (20 cm deep) open wooden box against the wall next to the door. The experimenter then left the room. The test started when the door closed, leaving the cat alone with the unfamiliar person. The test consisted of two parts. For the first three minutes the unfamiliar volunteer sat cross-legged on the floor, looking at the wall and ignoring the cat however close it got. We used an approach score from 1 to 5 depending on whether the cat did the following: (1) remained outside the large circle; (2) entered at least its forepaws in the large circle; (3) entered at least its forepaws in the small circle; (4) established physical contact with the human (rub, sniff, touch with paw); (5) put at least its forepaws on top of the human. Then, in the second part, the volunteer continuously called the cat by its name for one minute while extending his arm and index finger as a greeting, pointing in the cat’s direction, even if the cat had already made physical contact with him. See Table 1 for definitions of the behaviours analysed in this test.

- Active human approach test

This test was performed immediately after the passive human approach test. The volunteer was instructed to slowly rise to his feet, approach the cat and attempt to stroke it six times from the head to the base of the tail. If the cat moved away before it could be stroked six times, the unfamiliar human walked after it and attempted to stroke it again. The test ended after the sixth stroking attempt (whether successful or unsuccessful) or after 1 min. The experimenter then entered the room and returned the cat to its home room.

2.4. Ethical Considerations

Throughout the study, animals were kept and treated according to the guidelines for the use of animals in research as published in Animal Behaviour (ABS, 2016), as well as the relevant legislation for Mexico (National Guide for the Production, Care and Use of Laboratory Animals, Norma Oficial Mexicana NOM-062-200-1999), and approved by the Institutional Committee for the Care and Use of Laboratory Animals (CICUAL, permission ID 6315) of the Institute of Biomedical Research, UNAM, Mexico City, Mexico.

2.5. Video and Statistical Analysis

All behavioural variables were coded using Solomon Coder software for video analysis [60]. Statistical analyses of the data were carried out using the programme R, version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria) [61]. Prior to fixed-effects and repeatability analyses, any non-normally distributed continuous variables were normalized using either a Box–Cox or log transformation with the R package MASS [62]. Effects of sex, age and trial number on behavioural variables were analysed using linear mixed-effects models (LMM) for continuous, and generalized linear mixed-effects models (GLMM) for count (i.e., Poisson distributed) or binary (binomially distributed) dependent variables with the R package lme4 [63]. As fixed effects, we included sex, trial number (1 to 3), age (as a covariate), the interaction of sex \times age and the interaction of trial number \times age. As a random factor, we included individual identity. We applied backwards stepwise reduction of the full models beginning with non-significant interactions followed by non-significant fixed effects when $p > 0.05$. Individual identity as a random factor was retained in all models to account for repeated measures of individuals. p -values were extracted by Wald chi-squared tests (type III).

We then analysed the repeatability of individuals' behaviour across the three trials by intra-class correlations calculated as the proportion of phenotypic variation that can be attributed to between-subject variation [64]. We used GLMM-based calculations for count (Poisson distributed) or binary (binomially distributed) data and LMM-based calculations (Gaussian distributed) for continuous data for testing the repeatability of individual differences using the R package rptR [65,66]. Individual identity was used as a random factor and the fixed effects found to have a significant effect on each behaviour in the previous analysis were included where applicable. For all intra-class correlations, we calculated 95% confidence intervals by 1000 bootstrap steps, and p -values were calculated by 1000 permutations.

To investigate the possible association of the behaviours between the different tests, we first performed principal component analyses (PCAs) independently on each of the following tests: separation/confinement, mouse and passive human approach using spectral decomposition assuming correlation matrices, to reduce the number of dimensions; no rotations were used. In the case of the struggle and active human approach tests, we used the raw behavioural data, since only one behaviour was coded in each of these two tests. Since phenotypic correlations between traits may originate from two sources, that is, (i) from individuals' average levels of two traits (between-individual correlation) or (ii) from individuals' change in behaviour (within-individual correlation) [67–70], we calculated between-individual and within-individual (residual) correlations by using multivariate linear mixed models with the R package sommer [71] to partition possible phenotypic correlations between the traits. p -values were corrected for multiple tests using the Benjamini–Hochberg method.

3. Results

3.1. Repeatability of Individual Differences within Tests

3.1.1. Struggle Test

No effects of age, sex or trial or of the interaction between these were found on the latency to struggle (Supplementary Material Table S3). All cats ($n = 30$) struggled within the 30-s limit, with only one cat still held at 30 s on one occasion. Individual differences in the latency to struggle were significantly repeatable across the three trials (Table 2).

Table 2. Repeatability of the variables analysed for each of the behavioural tests. Intra-class correlation coefficients (R), 95% confidence intervals (lower bound, upper bound) based on 1000 bootstrap steps and significance values (p) are given. Asterisks indicate significance levels at $p < 0.05$ *, $p < 0.01$ **, $p < 0.001$ ***.

Behaviour	R	95% CI (lower bound, upper bound)	p -Value
Struggle test			
Latency to struggle	0.555	(0.314, 0.726)	0.001 ***
Separation/confinement test			
Latency to vocalize	0.761	(0.597, 0.861)	0.001 ***
Number of vocalizations	0.920	(0.766, 0.969)	0.006 **
Latency to motor activity	0.191	(0, 0.442)	0.066
Duration of motor activity	0.323	(0.06, 0.533)	0.001 ***
Mouse test			
Latency to approach	0.515	(0.248, 0.714)	0.001 ***
Duration near	0.498	(0.219, 0.679)	0.001 ***
Duration of tail swishing	0.806	(0.366, 0.944)	0.001 ***
Latency to interact	0.477	(0.201, 0.672)	0.001 ***
Duration interacting	0.501	(0.236, 0.716)	0.001 ***
Duration walking around	0.284	(0.019, 0.542)	0.017 *
Passive human approach test			
Approachscore (1–5)	0.312	(0, 0.507)	0.004 **
Latency to vocalize	0.668	(0.461, 0.806)	0.001 ***
Number of vocalizations	0.844	(0.632, 0.942)	0.008 **
Finger–nose contact (binary)	0.761	(0.376, 0.985)	0.001 ***
Active human approach test			
Stroking (latency)	0.496	(0.229, 0.692)	0.004 **

3.1.2. Separation/Confinement Test

Age and trial number (1–3) were found to have a small, significant effect on the number of vocalizations and the duration of motor activity; older cats vocalized less and moved less in the carrier, and both behaviours diminished in consecutive trials (Supplementary Material Table S3). In the case of latency to initiate motor activity, there was a significant but very small effect of sex, where males began motor activity slightly sooner. There was a small effect of the interaction between age and sex, where the latency to move was slightly higher in older males than in younger males. There was also a small effect of trial number, where latency to begin motor activity began slightly later in consecutive trials (Supplementary Material Table S3). Therefore, these significant fixed effects were included in the respective repeatability analyses. Individual differences in the latency to vocalize and the number of vocalizations emitted by the cats ($n = 28$) were highly repeatable. Duration of motor activity was also significantly repeatable, although the latency to locomote was not (Table 2).

3.1.3. Mouse Test

The sequence of trials was found to have an effect on the duration of interactions (cats interacted less with the mouse on the third trial than during the first two trials) and was thus added as a fixed effect in the analysis (Supplementary Material Table S3). No other variable showed an effect of age, trial number or sex or the interaction between them. We found highly repeatable individual differences in the latency to approach and the time cats ($n = 24$) spent near the mouse across trials. Variables associated with proximity to the mouse were likewise repeatable, such as the time spent walking around the jar, the latency to interact and the duration of interaction (Table 2). Even tail swishing, which was coded from any area of the room, showed repeatable individual differences, a possible sign of interest or arousal of the animal even from afar.

3.1.4. Human Approach Tests

None of the behavioural variables measured in these tests was significantly affected by age, trial number or sex or the interaction between them (Supplementary Material Table S3).

- Passive human approach test

We found repeatable individual differences ($n = 28$) for all behavioural measures in both phases of the test across trials, that is, the distance individual cats kept from the unfamiliar human was consistent even though each of the three trials used a different unfamiliar volunteer. We also found repeatable individual differences for the finger-nose contact measure of phase two. Moreover, individual differences in the latency to vocalize and in the number of vocalizations emitted during the entirety of trials were also highly repeatable (Table 2).

- Active human approach test

Individual differences in the latency for the unfamiliar person to be able to stroke the cat were consistent across trials and even though this involved three different people (Table 2).

3.2. Correlations Between Tests

For dimension reduction purposes, we performed three separate PCAs on the behavioural variables of the following tests: separation/confinement, mouse and passive human approach. For the full results of the PCAs, see Supplementary Material Table S4. In the separation/confinement test, two principal components were extracted. For factor 1 (“confinement/separation vocalization”), the behaviours with the highest loadings were those related to vocalization and, for factor 2 (“confinement/separation motor activity”), the highest loading was the duration of motor activity. In the mouse test, two principal components were extracted. For factor 1 (“interaction with the mouse”), the behaviours with the highest loadings were related to the cats’ proximity to and interaction with the mouse jar and, for factor 2 (“tail swishing”), the highest loading was for the duration of tail swishing. In the passive human approach test, two principal components were also extracted. For factor 1 (“approaching the passive human”), the behaviours with the highest loadings involved the human approach score and finger–nose contact; for factor 2 (“passive human approach vocalization”), the behaviour with the highest loading was the number of vocalizations.

In each of the two remaining tests (struggle and active human approach), we measured only one behavioural variable (latency to struggle and latency to be stroked by the human, respectively), hence we did not perform a PCA for these tests. Using the raw data for these variables, along with the six previously described factors obtained from the PCAs, we calculated correlations using multivariate linear mixed models. From a total of 34 correlations (Supplementary Material Table S5), we found eight that were significant after adjusting p -values for multiple comparisons (Benjamini–Hochberg method; Figure 1).

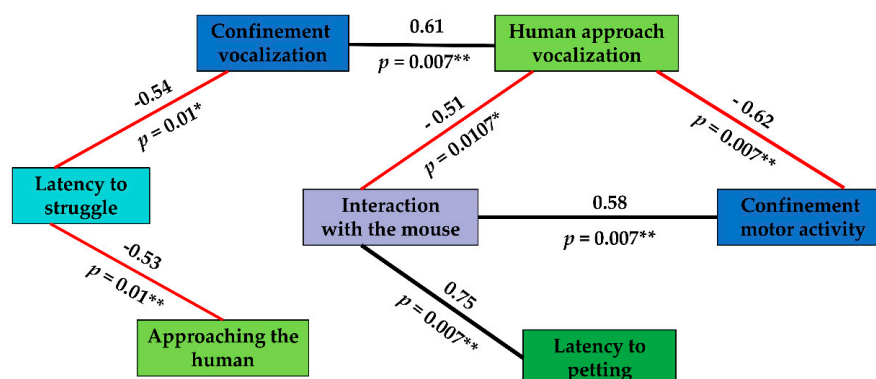


Figure 1. Correlations between behavioural variable scores showing stable individual differences at the between-individual level. Asterisks indicate significance levels at $p < 0.05$ *, $p < 0.01$ **. Black lines correspond to positive correlations, red lines correspond to negative correlations. Line thickness corresponds to the strength of a correlation. Further details are available in Supplementary Material Table S5, including confidence intervals.

4. Discussion

4.1. Consistency Across Time

In this study, we first evaluated the consistency across time of individual differences in behavioural responses of adult shelter cats in five different tests, and for all tests we found measures that showed significant repeatability. Stable individual differences were evident even though the cats were a heterogeneous population that differed in age, sex and (largely unknown) background. Perhaps surprisingly, individual differences in behavioural responses in most of the tests were unrelated to age or sex, suggesting that the behaviours measured here may be useful for evaluating individual differences in adult cats in general. This is supported by previous studies reporting stable individual differences in cats and other mammals in tests similar to those used here, that is, struggle or restraint tests used in cats [26], mice [31], rabbits [32,72,73], North American red squirrels [74,75] and pigs [33,76]; social separation tests used in cats [29,44,45], horses [77] cows [39,78] and dogs [40]; mouse tests used in cats [51,79]; and human approach tests used in cats [21,25,27,56,57,59], dogs [80], pigs and cattle [81,82]. These tests in their various forms are all relevant to the daily life of most cats, and thus provide a promising basis for assessing cat personality across a wide range of populations and conditions, including in shelter cats.

4.2. Behavioural Syndromes

We found seven significant correlations between behavioural scores from the different tests (Figure 1). Most of these seemed to be connected with humans; for example, cats that readily approached the unfamiliar human in the passive human approach test also struggled sooner in the struggle test, which may suggest that these cats were more confident around humans. Cats that struggled sooner also tended to vocalize (meow) more during the confinement test when separated from humans and other cats, suggesting that these individuals may seek the company of humans more, since meowing is considered a human-oriented behaviour ([83,84] our observation). Such correlations may indicate the existence of behavioural syndromes as defined in the Introduction.

We can suppose that while the separation/confinement test was probably a negative experience for all the cats, the human approach test was a positive experience for at least some individuals. A more detailed acoustic analysis of the meows may help disentangle the emotional valence and motivation (e.g., stress, attention-seeking, greeting) underlying them in these tests, since meows emitted during distress have a distinct pattern (low mean fundamental frequency, longer duration; Schötz et al. [85]). Additionally, the cats for which the human approach test was a positive experience may have emitted

other vocalizations (e.g., purrs, which Fermo et al. [86] found are exclusively associated with positive experiences). However, we were not able to record them due to their low volume. It is also possible, as Guillette and Sturdy [87] have suggested, that the degree of arousal or readiness of the cat to act (due to activation of the sympathetic nervous system) may contribute to the pattern of vocal emissions in different contexts [88].

Consistent with previous findings, we did not find an association between the number of vocalizations and motor activity within the confinement/separation test, suggesting different underlying mechanisms (motivation) between these variables (see more details in [29]). However, there was a negative correlation between motor activity in the confinement/separation test and the number of vocalizations emitted during the passive human approach test. The only explanation we can presently offer is that the cats for which the passive human approach test was a positive experience may have “carried” this correlation, meaning that possibly only positive meows are correlated with motor activity. Further study into the relationship between meows and motor activity in positive and negative situations may help to disentangle this.

Additionally, interaction with the mouse was significantly correlated with three different variables. It was negatively correlated with vocalization in the human approach passive phase, which can be interpreted as cats that were more focused on the mouse were less demanding of human attention (vocalized less). The latter is supported by the positive correlation between interaction with the mouse and latency to be stroked in the active human approach test, i.e., cats that spent more time with the mouse took longer to allow themselves to be stroked. Taken together, these correlations suggest a syndrome where more prey-oriented individuals are also less human-oriented. Although cats’ backgrounds in the present study were unknown, we speculate that such a syndrome may arise as a consequence of experiences prior to their arrival at the shelter, that is, cats that were more independent from humans may have relied more on hunting to obtain food, whereas cats that were more social with humans had relied on them for sustenance. Finally, there was also a positive correlation between interaction with the mouse and motor activity during the confinement/separation test, suggesting that some cats were more “excitable” than others, possibly due to differences in sympathetic nervous system arousal as discussed previously for vocalizations.

4.3. Behavioural Testing in Animal Shelters

All five tests implemented in this study are simple and fast (no more than five minutes each), and any materials used are inexpensive and easily procured. Because of this, they can be reproduced practically anywhere in the world with minimal instruction of shelter personnel. Together, this makes them a suitable option for shelters looking to evaluate personality as part of their adoption programme. While millions of cats enter animal shelters every year, in the United States, for example, only an estimated 11.5% of pet cats come from a shelter [4,28]. Furthermore, even if a cat is adopted, there is still a high chance that it will be returned due to not fulfilling the new owner’s expectations, which risks euthanasia. Organizations like the American Society for the Prevention of Cruelty to Animals have managed to decrease the number of returned cats by applying questionnaires and personality tests [28].

However, these protocols are not applied worldwide, due to differences in owner expectations and the way shelters operate in different locations, among others. For example, animal shelter facilities in Mexico and throughout Latin America differ from those in the United States and Europe, something also noted by Fukimoto et al. [89] in their study of shelter cats in Brazil. Although our tests share some similarities with the ASPCA’s Feline-ality behavioural assessment, we sought to develop tests that could be a better fit for the shelter conditions and owner expectations we are familiar with. For example, we chose to use the pet carrier as a test within itself to evaluate individual responses to isolation and confinement, as separation anxiety is a common concern for owners who work long hours away from home. We also included a novel test (mouse test) in which the cats are presented with a biologically relevant stimulus. Although we recognize that this test will not be relevant to all cats that are offered

for adoption as pets, nor is it feasible for all shelters to keep mice for this test, we would like to note that. In some shelters around the world, there are programmes to adopt out or loan “mouser” or “barn” cats ([c.f. [90] and also see the programs of the following organizations: Battersea Dogs & Cats Home (UK), Dereham Adoption Center (UK), Animal Humane Society (USA), Best Friends Animal Society (USA), Barn Cats Inc. (USA), among others). In recent years, there has been an increase in the demand for mousers by more environmentally friendly businesses and organic farms seeking to avoid rodenticides and to switch to biological pest control. This is an option for cats that are not sociable with people. Those individuals that show a strong interest in potential prey probably have a better chance of being successfully adopted into a working context.

Implementing repeated behavioural testing in the adoption process, whenever possible, could help match prospective owners with an animal that best suits the needs and lifestyle of both parties. For example, a family with small children needs a cat that tolerates handling; a calm person may want a calm cat; and someone who is not home most of the day would do better with a cat that is not stressed by separation.

5. Conclusions

Reliable, economic and easily implemented behavioural tests are needed by animal shelters to improve their adoption programmes by improving the match between the personality of the prospective pet, in this case the cat, and the context of its new home. This can be best achieved by using tests based on the natural, evolved behaviour of the cat relevant to its everyday life and using correlations between more than one behavioural measure to form a more reliable profile of each individual cat’s personality. Results of the present study indicate that this is, indeed, feasible.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2076-2615/10/6/962/s1>, Table S1: Details of the subjects and test assignment, File S2: Description of the housing of the mice during the experiments, Table S3: Results of the LMMs and GLMMs to evaluate the impact of age, sex and trial number on behaviour, Table S4: Full results of the PCAs performed on the confinement/separation, mouse and passive human approach tests, Table S5: Correlations between behavioural variable scores showing stable individual differences.

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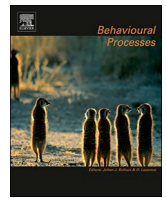
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Anexo II

Diferencias individuales estables en la vocalización y actividad durante estrés agudo en el gato doméstico

Stable individual differences in vocalisation and motor activity during acute stress in the domestic cat

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Stable individual differences in vocalisation and motor activity during acute stress in the domestic cat

Andrea Urrutia^{a,b,*}, Sandra Martínez-Byer^a, Péter Szenczi^{c,d,e}, Robyn Hudson^a, Oxána Bánszegi^{a,*}

^a Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, AP 70228, CP 04510, Mexico City, Mexico

^b Posgrado en Ciencias Biológicas, Unidad de Posgrado, Edificio A, 1er Piso, Circuito de Posgrados, Ciudad Universitaria, Coyoacán, CP 04510, Mexico City, Mexico

^c Cátedras CONACyT, Consejo Nacional de Ciencia y Tecnología, Mexico City, Mexico

^d Dirección de Neurociencias, Instituto Nacional de Psiquiatría Ramón de la Fuente Muñiz, Mexico City, Mexico

^e Unidad Psicopatología y Desarrollo, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico City, Mexico

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ABSTRACT

The behavioural assessment of individual animals in stressful situations should consider measures which are consistent across repeated testing, and therefore truly representative of an individual's behaviour. Here we report a study conducted on 40 neutered adult cats (*Felis silvestris catus*) of both sexes, originating from two animal shelters in Mexico and Hungary. We recorded the responses of the cats to repeated brief confinement trials that mimicked a common situation (confinement in a pet carrier). This test was repeated three times, leaving one week between trials, to assess short-term repeatability. Stable inter-individual differences in two behavioural measures, the number of separation calls and the duration of motor activity, were found, although the inter-individual differences in vocalisation were more pronounced than they were for motor activity. Additionally, the overall number of vocalisations emitted remained stable despite repeated testing, whereas motor activity tended to decrease week to week. There was a negative effect of age on vocalisation rate, and no effect of sex on either behaviour. No correlation between the two behavioural measures was found. We suggest that, in adult cats, vocalisation may be more reliable than motor activity as a behavioural measure of stress.

1. Introduction

The domestic cat (*Felis silvestris catus*) is currently one of the most popular companion animals in the world (American Pet Products Association, 2017; The European Pet Food Industry Federation, 2017). The need to understand its behaviour, cognition and cat-human relations is reflected in special issues and reviews in scientific journals dedicated to these topics in recent years (special issues: Farnworth, 2015; Udell and Vitale Shreve, 2017; reviews: Gartner, 2015; Gartner and Weiss, 2013; Litchfield et al., 2017). Consistent individual differences in animal behaviour (also known as personality) have attracted increasing attention in this regard as a research topic in recent years. The domestic cat is a good model species for such studies due to its rich behavioural repertoire and worldwide availability. In fact, in this field it is by far the most studied feline species, through observation, surveys, and a variety of behavioural tests, such as novel object, handling, and human approach tests (Gartner, 2015; Gartner and Weiss, 2013)

A key interest in the study of individual variation is the stability of

individuals' responses to stressful situations (Koolhaas et al., 2007). Separation tests are useful to measure individual differences in behavioural responses to stress in several mammalian species (review: Forkman et al., 2007) e.g. in pigs (Friel et al., 2016; Leliveld et al., 2017), horses (Merkies et al., 2016; Seaman et al., 2002), cattle (Watts et al., 2001), goats (Nawroth et al., 2017) and cats (Hudson et al., 2017, 2015; Iki et al., 2011; Yeon et al., 2011). Separation, or isolation, calls and motor activity are two behaviours which are most commonly used to study stress response since both tend to increase with arousal (Kiley, 1972; Rushen, 2000). Vocalisation reflects the inner, emotional state of the caller (Briefer, 2012; Jürgens, 2009; review: Newman, 2007), and in many mammalian species it is relatively easy to elicit calls through social separation or isolation, e.g. cattle (Boissy and Le Neindre, 1997), cheetahs (Ruiz-Miranda et al., 1998), primates (e.g. Norcross and Newman, 1997), even in species which are not considered to be highly social, such as giraffes (Tarou et al., 2000) and cats (Iki et al., 2011; Yeon et al., 2011). Note, however, that response to extreme stress can elicit freezing behaviour in many species (e.g. Brandão et al., 2008).

* Corresponding authors at: Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, AP 70228, CP 04510, Mexico City, Mexico.

E-mail addresses: andreaurrutia@outlook.com, andreacitla@comunidad.unam.mx (A. Urrutia), brownie_byer@ciencias.unam.mx (S. Martínez-Byer), peter.szenczi@gmail.com (P. Szenczi), rhudson@biomedicas.unam.mx (R. Hudson), oxana.banszegi@gmail.com (O. Bánszegi).

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In a previous study on cats, [Lki et al \(2011\)](#) found that adult males showed a strong negative correlation between locomotion and vocalisation behaviour during a stressful situation. However, in that study the cats were tested only once, so there is no information presently available on the stability of these behavioural responses across repeated testing in adults. In kittens, however, the stability of these responses has been evaluated through brief separation trials repeated once a week for the first four postnatal weeks, finding stable individual differences in the frequency of emitting separation calls and in locomotion. Nevertheless, there was no correlation between the two variables, possibly due to differential maturation of the vocal and motor systems in young kittens ([Hudson et al., 2015](#)). Like many other altricial mammals, kittens emit separation calls starting immediately after birth and the number of these increases during the first postnatal month, after which time there is a marked decrease ([Bánszegi et al., 2017](#)). Locomotor activity, on the other hand, develops more slowly over several months ([Levine et al., 1980](#); [Peters, 1983](#); [Villablanca and Olmstead, 1979](#)).

The ability to reliably assess the behaviour of individual animals in stressful situations using simple, practical and rapid assays would be beneficial in many situations involving farmed, companion and working animals, e.g. during veterinary visits ([Pratsch et al., 2018](#)). Individual differences in stress response to an aversive situation (e.g. transport in a pet carrier) are important, as they can make handling difficult and also affect the outcome of examinations and medical diagnoses. For example, in cats, symptoms of stress such as altered heart and breathing rate, elevated blood pressure or drooling can either confound or be confused with symptoms of disease, potentially affecting diagnosis ([Pratsch et al., 2018](#)). Good assessment tools should include not only species-adequate test design, but also the selection of behavioural measures which are consistent across repeated testing, and therefore truly representative of an individual's behaviour. In many species, vocalisation rate during isolation or confinement is repeatable and can be easily and reliably measured even in short tests, e.g. in sheep ([Wolf et al., 2008](#)) and pigs ([Friel et al., 2016](#)), including in kittens of the domestic cat ([Hudson et al., 2017, 2015](#)). We are interested in knowing if this pattern is found in adult cats as well, and how quickly in a test individual differences can be found.

It was therefore our aim in the present study to investigate the existence of stable individual differences in the behaviour of adult cats from a heterogeneous population. We recorded individuals' responses to brief social separation on repeated occasions using two behavioural measures: the number of separation calls and the duration of motor activity. We conducted a fine analysis of the data asking the following questions: (i) How stable are individual differences in cats in number of separation calls and duration of motor activity in response to brief social isolation across repeated trials? (ii) How quickly and reliably do any such differences emerge within trials? (iii) Does the expression of these behaviours change with repeated testing? (iv) Is there a relation between the two behavioural measures?

2. Material and methods

2.1. Study sites and animals

We collected data from 40 neutered adult cats between the ages of 8 months (after the age of sexual maturation) and 11 years (mean: 3.92 ± 2.4 SD years) from two different animal shelters. Twenty-nine cats (16 female, 13male) were housed at an animal shelter in Mexico City, Mexico and 11 (8 female, 3male) were housed in an animal shelter in Budapest, Hungary. Animals were chosen based on age, good health, ease of handling and remaining in the shelter for the duration of the study.

Both shelters were furnished with cat beds, boxes of assorted sizes with blankets, scratchers, and toys. Water, commercial dried cat food and litter boxes were always available. All the cats had been neutered and received post-operative care by qualified veterinarians within 3

days of entering a shelter, and all subjects participating in the study had been at the shelter for at least 6 weeks prior to the behavioural testing. In both shelters, cats were regularly exposed to and handled by volunteers and staff.

In the Hungarian cat shelter, the total number of cats fluctuated due to cats arriving and leaving, but at any given time approximately 20 cats were housed there. They were in a room 4 x 4 m in size connected to a fenced and roofed outdoor enclosure 3 x 3 m in size. The Mexican shelter was a 4-story house divided into sections; approximately 50 cats were placed in each section, according to how well they tolerated each other. All sections consisted of at least two rooms (approx. 2.5 x 3.5 m each) with access to a fenced outdoor balcony (approx. 2 x 4 m). Each cat was free to roam within its section.

2.2. Experimental procedure

At each site, the confinement trials were performed in a single closed room which was unfamiliar to the animals, located in the same building where they were housed and no more than 10 m away from the rooms that they typically occupied. In the Hungarian shelter, the testing room was 3 x 4 m in size, with a white tiled floor and walls and white ceiling, furnished with steel counters, and with a curtained window; in the Mexican shelter, the testing room was 1.5 x 2 m in size, with a flat finished, unpainted concrete floor, walls and ceiling, and without furnishings. Both rooms were illuminated by electric light. During trials, no other animals or humans were allowed to enter the test room nor the room adjacent to it to reduce potential auditory and olfactory disturbance. For each trial, all cats from the same shelter were tested on the same day in a randomized order between 13:00 h and 18:00 h once a week for three consecutive weeks.

One experimenter chose a cat from the housing area, briefly stroked it and picked it up. The experimenter then carried the cat, in their arms, into the test room. This procedure typically took less than one minute, and cats rarely vocalised during it. Once inside the test room, the first experimenter, with the help of a second experimenter, placed the cat inside a standard commercial pet carrier (42 × 61 x 38 cm), which was a closed plastic box with a steel-wire front door, and small ventilation holes along the walls. The carrier, with the cat inside, was then placed facing away from the door, after which the experimenters left the room and the trial began. The confinement test lasted two minutes; once this time had elapsed the cat was released from the pet carrier and returned to its home room carried in the arms of the first experimenter. All trials were recorded with a video camera (GoPro© Hero3+, GoPro, Inc., USA) which was set up 60 cm from the carrier allowing a clear view of its interior. To facilitate observation, a red light was mounted on the ceiling of the carrier. The carrier was cleaned between trials with isopropyl alcohol.

2.3. Behavioural recording and analysis

Using Solomon Coder software ([Péter, 2015](#)), the following behaviours were scored for each cat from the video recordings: vocalisation (the timing and total number of calls emitted by each individual during each separation trial) and duration of motor activity (displacement of any of the limbs on the floor or on the sides of the carrier; quantified in seconds at intervals of 0.5 s by analysis of video footage for each individual in each trial).

2.4. Statistical analysis

Statistical analyses of the data were carried out using the program R, version 3.3.1 ([R Development Core Team, 2016](#)). To assess inter-observer reliability, two observers independently scored the cats' behaviour for a subset of 30 trials (25% of the total), from 5 and 17 cats from the Hungarian and Mexican shelters, respectively. Inter-observer reliability was assessed using weighted Cohen's Kappa for vocalisation

data and a one-way intraclass coefficient (ICC) for motor activity data using the R package *irr* (Gamer et al., 2013). Agreement between the two independent observers was high both for number of vocalisations (weighted $\kappa = 0.962$, $p < 0.001$) and duration of motor activity ($ICC = 0.891$, $p < 0.001$).

Prior to analysis, motor activity was normalized using a Box-Cox transformation (Shapiro-Wilk test of normality: $W = 0.98$, $p = 0.084$) using the R package *MASS* (Venables and Ripley, 2002). We tested for significant effects of the experimental setup (shelter of origin), and of individual characteristics of the cats (age, sex and their interaction) on each of the two measured behaviours (vocalisation and motor activity) by fitting generalized linear mixed models (GLMMs) using the *lme4* package (Bates et al., 2015) in R. For each behavioural measure, we included the following variables into the full model. As fixed effects, we included sex, shelter (in Mexico or Hungary), trial number (1–3), age (as a covariate), the interaction of sex \times age, and the interaction of trial number \times age. As a random factor, we included individual identity. We applied backward stepwise reduction of the full models beginning with non-significant interactions followed by non-significant fixed effects when $p > 0.05$. Individual identity as a random factor was included in all models to account for repeated measures of individuals.

Across-trial and within-trial repeatability, that is, the amount of variance explained by the individual divided by the total phenotypic variance (Sokal and Rohlf, 1995), of each of the two behaviours (number of vocalisations and transformed duration of motor activity) was calculated using intra-class correlations obtained through GLMM-based calculations for count (Poisson-distributed) and LLM-based calculations for normalized data, respectively, using the R package *rptR* (Nakagawa and Schielzeth, 2010). Individual identity was always used as a random factor, and the fixed effects found to have a significant effect in the previous analysis were included where applicable. *P*-values were calculated by 1000 permutations and 95% confidence intervals (95% CI) for parameter estimates were assessed by 1000 bootstrapping runs. A variance decomposition analysis, where the proportions of the total variance in the expression of the two behavioural variables due to within-individual variance and between-individual variance, was also carried out using the *rptR* package.

To test for an association between the number of vocalisations and the duration of motor activity, mixed models separating between-individual and within-individual slopes were used. Since one behavioural variable must be handled as a predictor and the other as a response, two models were built: one where number of vocalisations was the response variable, and another where (normalized) duration of motor activity was the response variable. In each model, the other behavioural variable used as a predictor was included in two ways: averaged by individual (to eliminate within-subject variation and examine between-individual variation) and centred within subjects (to eliminate between-individual variation and examine only within-individual variation; van de Pol and Wright, 2009). Also included as fixed effects were sex, shelter of origin, trial number and age, as well as the interactions between each of these fixed effect and the behavioural predictor variables (both averaged by individual and within-subject centred); individual identity was included as a random effect. We applied backward stepwise reduction of the full models beginning with non-significant interactions followed by non-significant fixed effects when $p > 0.05$. Individual identity as a random factor was included in all models to account for repeated measures of individuals.

2.5. Ethics note

Throughout the study, animals were kept and treated according to the guidelines for the use of animals in research as published in Animal Behaviour (ABS, 2016), as well as the relevant legislation for Mexico (National Guide for the Production, Care and Use of Laboratory Animals, Norma Oficial Mexicana NOM-062-200-1999) and Hungary

(European Communities Council Directive of 22 September 2010, 2010/63/EU).

3. Results

3.1. Repeatability of individual differences in behaviour

Age was found to have an effect on the number of vocalisations (Appendix Table A1), where older cats vocalised less. No effects of sex, trial number, the interaction of age \times sex, the interaction of trial \times sex, nor the shelter to which each cat belonged were found on the number of vocalisations during the study (Appendix Table A1). Therefore, age was included in the repeatability analysis as a fixed effect in addition to individual identity as a random factor. Individual differences in the number of vocalisations emitted by the cats ($n = 40$) across the three trials of the confinement test were highly repeatable (intra-class repeatability: $R = 0.913$, $CI = [0.803, 0.964]$, $p = 0.001$). Average between-individual variance ($3.826 \pm 1.106 SE$) was higher than the average within-individual variance ($0.386 \pm 0.099 SE$).

Trial number (one to three) had a negative effect on the duration of motor activity; no effects of age, sex, the interaction of age \times sex, the interaction of trial \times sex, nor the shelter to which each cat belonged were found on the duration of motor activity during the study (Appendix Table A2). Therefore, in the repeatability analysis, only trial number was included as a fixed factor and individual identity was included as a random factor. Although individual differences in motor activity ($n = 40$) were repeatable across trials, the repeatability value was low ($R = 0.423$, $CI = [0.209, 0.601]$, $p = 0.001$), because the average between-individual variance ($0.254 \pm 0.085 SE$) was lower than the average within-individual variance ($0.347 \pm 0.056 SE$).

3.2. Emergence of individual differences in behaviour during the trials

In regard to our second aim, which was to see how quickly individual differences emerged during trials, inter-individual differences in the number of vocalisations and the duration of motor activity were already significantly repeatable between trials after the first 10 s of testing (vocalisation: $R = 0.78$, $CI = [0.400, 0.949]$, $p = 0.001$; motor activity: $R = 0.226$, $CI = [0.019, 0.423]$, $p = 0.013$; Fig. 1). The repeatability of the number of vocalisations increased slightly after the first 20 s and remained stable until the end of the test, whereas the repeatability of motor activity increased slowly until ~90 s into the test, whereafter it remained stable (Fig. 1).

3.3. Overall change in performance of behaviours with repeated testing

In regard to our third aim, which was to determine whether the expression of the behaviours changed with repeated testing, we found that the number of vocalisations emitted did not vary between trials (Appendix Table A1), but the duration of motor activity did (estimate $\pm SE = -0.237 \pm 0.066$, $p = 0.0005$; Appendix Table A2). Paired Bonferroni post-hoc tests showed that motor activity decreased after the second trial (Fig. 2).

3.4. Correlation between vocalisation and motor activity

In regard to our fourth aim, the two models we constructed gave similar results (Appendix Table A3). There was no significant correlation in either of the models between the number of vocalisations and the duration of motor activity at the between-individual level. Although in one of the models (with duration of motor activity as the response variable) the *p*-values indicated that there was a significant relationship between the two behaviours at the within-individual level, the model estimates were extremely small (0.024 ± 0.01), so we do not interpret this result as indicative of a meaningful relationship between the

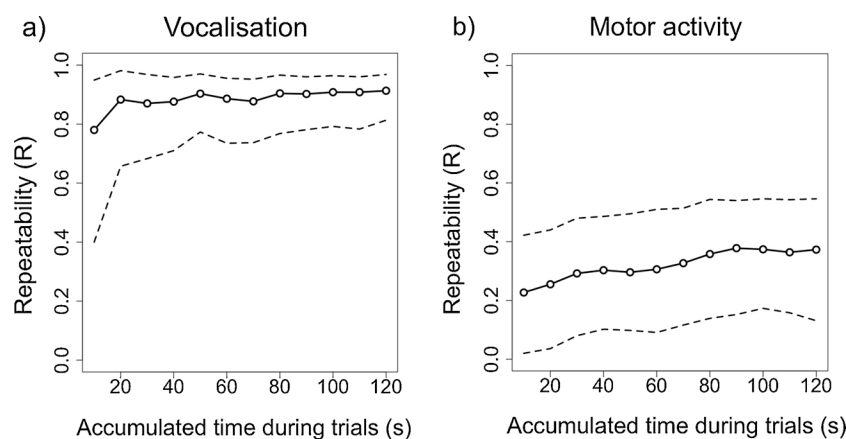


Fig. 1. Progression of repeatabilities of (a) the number of vocalisations and (b) the duration of motor activity of individual cats across the three, 2-min trials carried out over three weeks. Analyses are based on cumulatively increasing 10-second-long segments of the three trials of the study. Circles represent the intra-class correlation coefficient (R), expressing the degree of repeatability across the three trials. Dotted lines give the 95% confidence intervals of R , based on 1000 bootstraps. All R values in (a) and (b) are statistically significant.

variables. Likewise, although in the model where the number of vocalisations was the response variable the interaction of age * duration of locomotion (within-subject centred) was significant with a model estimate of -0.004 ± 0.002 , and the interaction of trial * duration of motor activity (within-subject centred) was significant with a model estimate of 0.012 ± 0.005 , the model estimates were again so small that we do not interpret these results as meaningful. The same is true in the model in which duration of motor activity was the response variable, where the interaction between trial * number of vocalisations (averaged by individual) was significant with a model estimate of 0.012 ± 0.005 , which again is extremely small, and we do not interpret this as a meaningful result.

4. Discussion

4.1. Stable inter-individual differences

The cats in this study showed high stable inter-individual differences in the number of vocalisations emitted during repeated periods of brief confinement, and to a higher degree than was previously found in kittens (Hudson et al., 2017, 2015). Stable differences in vocalisation have also been reported for adult cats in different test situations, e.g. in novel environments (Adamec et al., 1983). The duration of motor activity, on the other hand, showed significant but lower repeatability between trials; the average within-individual variance was greater than the average between-individual variance. The repeatability of motor activity in adult cats that we report here is lower than that of locomotor activity reported in kittens in a similar test situation (Hudson et al., 2017), suggesting that there could be an age-related change in inter-

individual differences in activity, although whether these differences were due to developmental, experiential or environmental factors cannot be determined without further study.

4.2. Change in inter-individual differences during trials

The repeatability of the number of vocalisations was high even after the first 10 s of the trials, and remained high thereafter which is again consistent with findings in kittens (Hudson et al., 2017), although the repeatability values in adults were higher than those in kittens. This is perhaps not surprising, since kittens' vocalisation in response to social separation changes rapidly in early age, which can drive down repeatability (Bánszegi et al., 2017). Repeatability of the duration of motor activity in the present study, on the other hand, was low in the first 10 s of the trial, and after a slow increase it appeared to plateau (at a low value) after 90 s, suggesting that even in a longer trial it would not become highly repeatable. This was different to the pattern observed in kittens, where repeatability of locomotor activity increased markedly until ~100 s into trials and then dropped (Hudson et al., 2017).

4.3. Change in behaviour with repeated testing

Previous works by Adamec et al. (1983) and Candland and Nagy (1969) showed that upon repeated testing, cats vocalised somewhat less frequently in later trials. However, those tests were 15 and 10 min long, respectively, and in the case of Adamec et al. (1983), this decrease was observed only after the first 8 min of the test. In the present study the cats were confined for only 2 min, which perhaps was not long enough

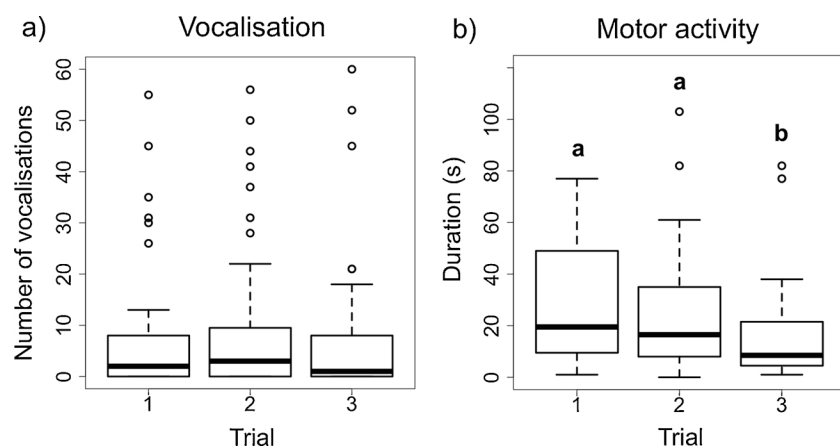


Fig. 2. a) The number of vocalisations emitted during the trials remained stable across repeated testing. b) The duration of motor activity decreased with repeated testing. Each boxplot depicts medians with inter-quartile ranges; whiskers extend to maximum 1.5 times the inter-quartile range. Different letters above boxes indicate significant differences following paired Bonferroni post-hoc tests.

to see a general change in the number of vocalisations week to week, since a decline in vocalisations has only been observed in long (> 10 min) tests. However, we did find a general decrease in the duration of motor activity over the three weeks of testing, supporting the findings of Candland and Nagy (1969) regarding activity. This general decrease in activity could be interpreted in different ways. One possible interpretation is that the animals habituated to the test, although this is an unlikely explanation since habituation should then also have been seen in the rate of vocalisations, which, however, remained stable from week to week. Another possibility is that the cats' motor activity was expressed in response to a combination of different motivations which changed with repeated testing, e.g. exploration or general activity.

4.4. Lack of correlation between number of separation calls and duration of motor activity

In kittens tested repeatedly from 1 to 4 weeks of age, no correlation was found between the number of vocalisations and amount of locomotion in an isolation test by Hudson et al. (2015), who suggested that this could be due to different rates of maturation of the locomotor and vocal systems. However, even in the fully mature cats used in the present study, no such correlation was found. This is in contrast to the results of Iki et al. (2011), who found a strong negative correlation between locomotion and vocalisation in adult male cats during a spray shower. The different results could be due to the characteristics of the study population (degree of socialization to humans and/or conspecifics, population density, housing conditions, or sex) or to characteristics of the negative stimulus: the motivation to move during confinement may differ from that during a spray shower.

The lack of correlation between motor activity and vocalisation is consistent with other reports in cattle (Van Reenen et al., 2004, 2005; Van Reenen et al., 2013), where it was suggested that the two behaviours could reflect two different underlying traits. Higher levels of vocalisation might be due to some individuals' stronger reactions to isolation or confinement, possibly due to the social aspect of the isolation (Müller and Schrader, 2005) or as a general fearful reaction (De Passillé et al., 1995), whereas high activity could be due to the animals having an active, rather than passive coping style in stressful situations (Koolhaas et al., 1999; Van Reenen et al., 2013). A similar explanation has been proposed by Iki et al. (2011) for individual differences in locomotion and vocalisation in cats exposed to a stressful situation. Alternatively, Van Reenen et al. (2013) also propose that locomotion may not be linked to an emotional reaction, but rather that it could reflect a general measure of activity or exploration, or (what seems more likely) that it cannot be exclusively attributed to a single underlying trait (Rushen, 2000). Due to the lack of correlation between the two variables in our results, we propose that in adult cats, and as considered previously in kittens (Hudson et al., 2015), they are due to (at least) two different neurophysiological mechanisms.

4.5. General discussion

Activity is one of the most common behavioural responses measured in stressful test situations and has been found to be repeatable in a variety of species, although its repeatability tends to be low (Bell et al., 2009). Additionally, whether high levels of activity actually indicate stress has been questioned (Rushen, 2000). It has been suggested that whether activity accurately reflects stress is most likely species-specific (Candland and Nagy, 1969), and most likely depends on the set-up of a particular test as well. The number or rate of vocalisation is less commonly used, although in several species it has also been found to be a good marker of stress and is repeatable, e.g. in cattle (Van Reenen et al., 2004; Van Reenen et al., 2005, 2013), pigs (Fraser, 1974), horses (Seaman et al., 2002), sheep (Torres-Hernandez and Hohenboken, 1979), kittens (Hudson et al., 2017, 2015), and adult cats in the present

study. In addition, some vocalisations have been linked to negative emotions (Brudzynski, 2007; Jürgens, 2002; Newman, 2007), as both vocal and physiological responses to stress are partially controlled by the same central neuroendocrine systems (Jürgens, 2009).

Vocalisation is increasingly recognized as a useful way to quickly and reliably measure the emotional state of individuals, both in experimental settings and in applied situations. For example, distress calls have seen applied use in welfare (Grandin, 1998; Manteuffel et al., 2004) and in pharmacological studies (Brudzynski, 2015). Although current methods of behavioural stress assessment in cats include vocalisation, such as the Cat-Stress-Score (CSS; Kessler and Turner, 1997) which combines the assessment of posture, facial expression, and behaviour, the interpretation of vocalisation remains somewhat ambiguous. The CSS rates both the presence *and* absence of vocalisation as indicative of high stress in cats, which suggests that there may be individual differences in how cats react behaviourally to stress (though we would like to note that the CSS was developed to assess stress over a period of several days rather than in response to short-term, acute stressors). In other areas of research, however, vocalisation in cats is a promising candidate for the behavioural assessment of stress, although the relationship between the two in cats is still unclear: one recent study found that vocalisation during confinement in a pet carrier was reduced by the administration of trazodone, an antidepressant and anxiolytic drug (Stevens et al., 2016); whereas another study found the opposite effect, where vocalisation during isolation in a room increased with the administration of the anxiolytic drug diazepam (de Rivera et al., 2017). The validation of vocalisation behaviour as a behavioural indicator of stress with additional measures, such as physiological variables of stress response or qualitative vocal analysis, could make for faster, easier and non-invasive assessment of stress reactivity in cats and potentially other animals.

In the present paper, we cannot attribute the vocal response we observed in the cats solely to confinement, isolation or, for example, the exposure to a novel environment. To disentangle the effect of each of these conditions as stressors further research is needed. Additionally, the underlying motivation and expression of behavioural responses to stress may change over development: for example, very young kittens placed in a novel environment will still vocalise even in the presence of their mother or a littermate, whereas older kittens (approaching weaning age) placed in novel environments tend to vocalise much less (Bánszegi et al., 2017), and particularly if they are in the presence of a familiar conspecific (Rheingold and Eckerman, 1971). The interpretation of behavioural responses to stress must always be carried out with caution as they will usually reflect a mix of motivations, although vocalisation in many species has been experimentally linked to hypothalamic–pituitary–adrenal (HPA) axis activity and a stress response (Rushen, 2000).

4.6. Conclusions

We found stable inter-individual differences in adult cats' behavioural responses to repeated brief confinement tests. Vocalisation rate was highly repeatable and it remained stable from week to week, whereas the duration of motor activity had very low repeatability values and tended to decrease with repeated testing. Our findings suggest that, in cats, vocalisations may be more reliable than motor activity as indicators of stable differences between individuals, and potentially a better measure of stress response. An important next step is to carry out repeated measurements across development, as it is still unknown whether individual differences in these behaviours are repeatable across different life stages. Behaviours may change as animals grow and mature, especially in altricial species, as developing offspring acquire skills and experience, exhibit more adult-like behaviours, and become self-sufficient (Stamps and Groothuis, 2010; Trillmich and Hudson, 2011). Nevertheless, since individual differences in the rate of separation calls was highly repeatable in adult cats and was previously found

to be repeatable in kittens, and more so than levels of motor activity (Hudson et al., 2015), it appears to be a good behaviour to study personality across different life stages.

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Appendix A

Models to check for significant effects of covariates

Table A1

Models investigating the effect of study location and characteristics of the cats on the number of vocalisations. Bolded effects were significant and were thus later included in the repeatability analysis.

Vocalisation		
Effect	Estimate ± SE	<i>p</i>
age	−0.455 ± 0.163	0.005
age*sex	−0.073 ± 0.318	0.819
trial*sex	−0.132 ± 0.078	0.089
sex	−0.025 ± 0.684	0.970
trial	0.007 ± 0.038	0.849
shelter	−0.913 ± 0.772	0.237

Table A2

Models investigating the effect of study location and characteristics of the cats on the duration of motor activity (normalized). Bolded effects were significant and were thus later included in the repeatability analysis.

Motor activity		
Effect	Estimate ± SE	<i>p</i>
trial	−0.237 ± 0.066	0.0005
age*sex	−0.054 ± 0.086	0.531
trial*sex	0.107 ± 0.136	0.436
shelter	−0.084 ± 0.241	0.730
sex	−0.024 ± 0.202	0.906
age	−0.046 ± 0.040	0.257

Table A3

Mixed models resulting after backwards stepwise selection investigating the relationship between number of vocalisations and duration of motor activity at the between-individual level (with data averaged by individual) and within-individual level (data within-subject centred) with a) number of vocalisations, and b) duration of motor activity as the response variables, respectively. Individual identity was included as a random effect.

a) Number of vocalisations		
Effect	Estimate ± SE	<i>p</i>
duration of motor activity, within-subject centred	−0.001 ± 0.011	0.94
age	−0.457 ± 0.167	0.006
trial	0.025 ± 0.041	0.539
age * duration of motor activity, within-subject centred	−0.004 ± 0.002	0.037
trial * duration of motor activity, within-subject centred	0.012 ± 0.005	0.009
b) Duration of motor activity		
Effect	Estimate ± SE	<i>p</i>
number of vocalisations, averaged by individual	−0.019 ± 0.012	0.1
number of vocalisations, within-subject centred	0.024 ± 0.01	0.02
trial	−0.339 ± 0.073	0.00001
trial * number of vocalisations, averaged by individual	0.012 ± 0.005	0.013

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.beproc.2019.05.022>.

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