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Diferencias individuales en comportamiento y respuesta de estrés en perros

Memoria presentada por **Susana Le Brech**

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El Dr. Xavier Manteca Vilanova, catedrático del Departamento de Ciencia Animal y de los Alimentos de la Facultat de Veterinaria de la Universitat Autònoma de Barcelona.

CERTIFICA:

Que la memoria titulada "**Diferencias individuales en comportamiento y respuesta de estrés en perros**", presentada por **Susana Le Brech** para optar por el grado de Doctor en Producción Animal, se ha realizado bajo su dirección y, considerándola acabada, autorizo su presentación para que sea juzgada por la comisión correspondiente.

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El director La doctoranda

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INTRODUCCION GENERAL

1. Diferencias individuales en comportamiento: aspectos generales

Las variaciones en comportamiento son evidentes en todas las poblaciones de perros (Jones y Gosling, 2005; Diederich y Giffroy, 2006), incluyendo aquéllas que han sido seleccionadas y criadas para un trabajo particular y que, por lo tanto, requieren la presentación de conductas determinadas (Willis, 1995; Graham y Gosling, 2009).

El término diferencias individuales se refiere a las diferencias en respuestas conductuales que son consistentes a lo largo del tiempo y entre contextos (Manteca y Deag, 1993; Koolhass, 2008), que caracterizan a ciertos individuos y que los distinguen de otros de su misma especie (Mendl y Harcourt, 2000). El estudio de estas diferencias intenta explicar los factores que predisponen a los individuos de una determinada especie a responder de maneras diferentes cuando son enfrentados a un estímulo similar. Por ejemplo, existen individuos que frente a un estímulo amenazante, reaccionan con agresividad enfrentando a dicho estímulo, mientras que otros reaccionan más pasivamente intentando evitarlo. En ratones, por ejemplo, dos líneas genéticamente diferentes fueron seleccionadas por presentar una latencia baja para atacar (BLA) o una latencia alta para atacar (ALA). Cuando se evaluaba la conducta agresiva se observaba que los machos BLA eran considerablemente más agresivos que los ALA (Oortmerssen y Bakker, 1981). Las diferencias individuales en comportamiento han sido identificadas en un amplio número de especies domésticas como los bovinos (Boissy y Bouissou, 1995), los perros (De Meester et al., 2008), los gatos (Feaver et al., 1986), los caprinos (Lyons, 1989) y los cerdos (Mason et al., 2003).

El estudio de las diferencias individuales ha mostrado un interés creciente dentro de la comunidad científica en los últimos años tanto en animales de compañía como en animales de producción. Uno de los motivos del creciente interés del su estudio es su relevancia para el bienestar animal. Así, por ejemplo, las diferencias individuales determinan, la capacidad de adaptación que tiene un individuo frente a una situación estresante (Koolhaas, 2008). El hecho de comprender las diferencias en la capacidad de adaptación es de suma importancia para el bienestar animal. Los animales domésticos, están sujetos a numerosos estímulos estresantes, muchas veces

relacionados con eventos impredecibles e incontrolables (Wiepkema y Koolhaas, 1993) siendo algunos individuos más vulnerables que otros a este tipo de estímulos.

Además, el hecho de comprender el origen y los mecanismos subyacentes de estas diferencias es muy importante. Así, por ejemplo, la identificación de estas diferencias puede ayudarnos a comprender los diferentes mecanismos causales o predisponentes de las mismas. La detección de dichos mecanismos, a su vez, es de primordial importancia práctica ya que, por un lado, puede ayudarnos a implementar protocolos de prevención destinados a favorecer la aparición de comportamientos aceptables desde el punto de vista del bienestar animal y de la convivencia con las personas. Por ejemplo, las diferencias de la respuesta frente a personas desconocidas en perros están influenciadas, al menos en parte, por el contacto que hayan tenido con las mismas durante etapas tempranas de la vida. Así, los perros que hayan tenido contacto frecuente con distintos tipos de personas durante el período de socialización, tendrán menos probabilidades de presentar problemas de miedo hacia personas desconocidas que aquéllos que hayan tenido poco contacto con personas durante este período (Beaver, 2009). Este conocimiento, permitiría diseñar por ejemplo un protocolo de socialización con personas en perros de laboratorio para facilitar su manejo por parte de personas disminuyendo el miedo hacia las mismas y así evitando un estrés innecesario en los animales.

Muchas de las conductas de interés para evaluar el bienestar, están relacionadas con una respuesta de estrés. Dicha respuesta, está relacionada a su vez con cambios fisiológicos que pueden evaluarse para detectar diferencias individuales. Así, cuando un animal se enfrenta a una situación de estrés se producen una serie de cambios, de los cuales, los más estudiados para la evaluación del bienestar animal son la activación del Sistema Nervioso Autónomo (SNA) y del eje Hipotálamo Pituitaria Adrenal (HPA) (Momèrde et al., 2007).

En perros, la hormona más importante en cuanto a evaluación del eje HPA en perros es el cortisol. Este glucocorticoide es sintetizado en la zona fascicular de la corteza adrenal bajo el control de la hormona pituitaria adrenocorticotropa (ACTH). La ACTH es sintetizada por células especializadas de la glándula pituitaria anterior y su liberación

se desencadena por la acción coordinada de dos neuropéptidos, la hormona liberadora de corticotropina (CRH) y la vasopresina (AVP) que son sintetizados por neuronas especializadas en el núcleo paraventricular del hipotálamo (PVN) y liberados en los capilares de la eminencia media por los que alcanzan la pituitaria directamente por la circulación hipotalámica pituitaria portal. Cuando el cortisol alcanza un nivel elevado en sangre, ejerce una retroalimentación negativa sobre el eje HPA, actuando sobre las células corticotropas de la pituitaria, el PVN y otras áreas del sistema nervioso central. De esta manera la actividad del eje vuelve a sus valores basales luego de la estimulación.

2. Factores causales de las diferencias individuales en conducta

Existen diversos factores que pueden predisponer a los individuos a presentar diferencias en conducta. Generalmente, la presentación de las diferencias individuales es el resultado de la interacción de factores genéticos y ambientales. En cuanto a los factores ambientales, son especialmente importantes, aquellos que se presentan en etapas tempranas de la vida.

2.1. Genética

La influencia de la genética sobre las diferencias individuales en respuestas conductuales y fisiológicas ha sido reconocida en numerosos estudios en roedores de laboratorio (Ramos et al., 1997; Durand et al., 1998; Muráni et al., 2010), ovejas (Boissy et al., 2005), gatos (Mc Cune, 1995; Marchei et al., 2011) y perros (Takeuchi et al., 2005; Ogata et al., 2006b).

En perros, una manera indirecta de evaluar diferencias genéticas es teniendo en cuenta las diferencias entre las distintas razas. Las diferencias entre razas deben analizarse e interpretarse con mucha precaución ya que no necesariamente se relacionan con diferencias genéticas (Manteca, 2003). Así, por ejemplo, las diferencias

entre razas pueden estar influenciadas por la percepción que tiene el evaluador sobre una determinada raza y que no necesariamente es acertada. Por otro lado, las diferencias entre razas pueden estar influidas por la personalidad de las personas que tienden a adoptar una determinada raza, los métodos de entrenamiento empleados y las formas de crianza de ciertas razas en particular (Amat et al., 2013).

En el estudio clásico realizado por Scott y Fuller (1965), se comparó la conducta de 5 razas de perros criados bajo las mismas condiciones y se observaron diferencias significativas entre razas que fueron atribuidas a factores genéticos. Se debe tener en cuenta, sin embrago, que se observó una importante variabilidad dentro de cada raza. En un estudio más reciente (Hart y Hart, 1985), se evaluó la conducta de 56 razas de perros por medio de puntuaciones adjudicadas por veterinarios y jueces de exposiciones caninas. Se observaron diferencias en los 13 rasgos de conducta evaluados. Una vez más, se observó una variabilidad importante entre individuos de la misma raza. Es necesario tener en cuenta, además, que el hecho de que este estudio se basa en puntuaciones adjudicadas por personas hace que sea más subjetivo y que, como dijimos más arriba, esas puntuaciones puedan estar sesgadas según la opinión personal de cada raza. En el caso particular de la raza Cocker spaniel Inglés se observó que la misma se encuentra más predispuesta a presentar agresividad de tipo impulsiva que otras razas de perros (Amat et al., 2009). Al parecer, esta diferencia se explicaría por la menor concentración de serotonina sérica que presentan los individuos agresivos de la raza Cocker spaniel Inglés en comparación con individuos agresivos de otras razas (Amat el at., 2013). Diferencias en el transporte, síntesis, liberación y metabolismo de serotonina han sido asociados con diferencias en conducta en personas (Courtet et al., 2001; Sukonick et al., 2001) y en perros (Reisner et al., 1996; Wright et al., 2012). Si bien en perros no se han detectado polimorfismos en este neurotransmisor, es probable que las diferencias sean genéticas (Amat et al., 2013). Sí se han detectado polimorfismos en genes involucrados en el control de otros neurotransmisores como la dopamina y glutamato (Niimi et al., 1999; Ogata et al., 2006b), los que explicarían algunas diferencias en conducta de perros.

2.2. Experiencias de la vida temprana.

El efecto del ambiente durante las etapas tempranas de la vida constituye uno de los factores más importantes que determinan la plasticidad fenotípica de los individuos (Koolhaas, 2008). Estos efectos son evidentes desde etapas tan tempranas como el período prenatal.

2.2.1. Período Prenatal

Los efectos del estrés materno sobre el feto han sido ampliamente estudiados en roedores de laboratorio y otras especies. El estrés prenatal puede afectar el desarrollo del feto debido a la exposición de hormonas de estrés que son transportadas a través de la placenta (Weinstock, 2008). Las hormonas de estrés que alcanzan el cerebro del feto desde la circulación materna, incluyen catecolaminas, CRH y glucocorticoides. Durante el estrés maternal, además se produce una constricción de las arterias placentarias, reduciendo el flujo sanguíneo fetal y por consiguiente el aporte de nutrientes esenciales y oxígeno, lo cual puede comprometer también su función y desarrollo fetal.

Se ha observado que ratas adultas que sufrieron estrés prenatal, presentan una mayor dificultad de adaptación a condiciones adversas que ratas control (Meijer, 1985). Así, dichas ratas, secretan más cortisol en respuesta al estrés psicológico que las ratas control. Otros estudios demuestran que las ratas que han sufrido estrés prenatal son más ansiosas que las ratas control cuando son expuestas a ambientes desconocidos como un test de open field (Poltyrev et al., 1996; Ward et al., 2000; Dickerson et al., 2005).

Las alteraciones asociadas con el estrés materno incluyen una alteración de la regulación del eje HPA, generalmente asociada con una hiperactividad de dicho eje y una alteración de los mecanismos de retroalimentación negativa. Además se produce una regulación decreciente de los receptores de glucocorticoides. Al parecer, estos efectos se producirían cuando el estrés maternal es de suficiente intensidad. Además,

las alteraciones producidas por el estrés materno en la cría, dependen en el estado de desarrollo de cada sistema neuronal particular y la presencia de receptores para glucocorticioides o mineralocorticoides en el momento del estrés (Braastad, 1998; Weinstock, 2008). Esto último varía en función de la especie.

Para nuestro conocimiento, no existen estudios experimentales sobre los efectos del estrés prenatal sobre la respuesta de estrés en perros. Sin embargo, en un estudio con zorros, se observó que las crías de madres que habían sido sujetas a una situación de estrés durante 1 minuto al día durante la gestación, presentaban una función adrenocortical aumentada y una mayor reactividad conductual en situaciones noveles que los individuos control (Braastad et al., 1998). Es probable que los resultados de este estudio así como los de los estudios realizados con roedores de laboratorio sean al menos parcialmente extrapolables a los perros.

2.2.2. Período Neonatal

Las experiencias durante la vida postnatal también pueden tener efectos sobre las diferencias individuales en comportamiento y respuesta de estrés a largo plazo. Este fenómeno es especialmente importante en especies altriciales como perros, gatos o ratas, cuyo grado de desarrollo en el momento del nacimiento es más inmaduro que el de las especies precociales (Manteca, 2003).

El efecto de la manipulación durante la vida neonatal, ha sido ampliamente documentado especialmente en roedores de laboratorio y ha demostrado tener efectos positivos sobre la capacidad de un organismo de adaptarse a una situación de estrés (Levine, 1957). Este fenómeno es el resultado de cambios epigenéticos que se modifican la función del eje HPA, entre otras estructuras. Así, al llegar a una edad adulta, las ratas que fueron manipuladas, exhiben una respuesta de miedo atenuada en ambientes nuevos y un menor incremento de la secreción adrenal de glucocorticoides en respuesta a una variedad de estímulos estresantes (Meaney et al., 1988).

La manipulación neonatal fue estudiada por primera vez en roedores de laboratorio (Levine, 1957). La misma consiste en separar al cachorro de su madre por un período corto de tiempo (de 5 a 15 minutos al día) (Levine, 1957; Levine, 1962; Levine et al., 1967). Cuando el cachorro es devuelto a su madre, la misma lo lame de manera más intensa que si el cachorro no hubiera sido separado. De esta manera, se incrementa la cantidad de estimulación táctil recibida por la cría (Priestnall, 1973) y es de hecho este efecto el que parece ser el principal responsable de modificar la respuesta de estrés el animal (Lay, 2000; Meaney, 2001).

Tal como lo comentamos más arriba, los efectos de la manipulación neonatal son mediados por la modificación epigenética de la expresión génica. La manipulación incrementa la expresión de los receptores de glucocorticoides (GR) en el hipocampo y corteza frontal (Meaney et al., 1985a), incrementando la sensibilidad de esas estructuras a los mecanismos de retroalimetación negativa del eje HPA a los elevados niveles de glucocorticoides circulantes e incrementando la eficacia de la inhibición neural sobre la secreción de ACTH (Meaney et al., 1985b; Meaney et al., 1991). Así, por ejemplo, Beane et al. (2002) observaron que las ratas que habían sido manipuladas durante la vida neonatal mostraban un retorno a los niveles basales de corticosterona más rápido que las ratas control luego de haber sido expuestas a una situación de estrés. En cuanto a la conducta, se ha observado que ratas que han sido manipuladas durante la vida neonatal muestran un incremento de la conducta ambulatoria y una menor frecuencia de defecación cuando son colocadas en un ambiente nuevo que animales control (Levine et al., 1967).

En perros, uno de los primeros estudios experimentales sobre la estimulación en etapas tempranas de la vida fue llevado a cabo por Fox y Stelzner (1966). En este estudio, los investigadores, expusieron a los cachorros a varios tipos de estímulos diferentes (visuales, auditivos, laberínticos, térmicos, manipulación, etc.) desde el primer día de vida hasta dos semanas pasado el período neonatal (5 semanas). Cuando los cachorros fueron evaluados a las 5 semanas de edad, se observaron diferencias significativas en su comportamiento, frecuencia cardiaca y actividad electrocardiográfica entre animales manipulados y no manipulados e interpretaron esas diferencias como positivas para los animales manipulados. A pesar de que el

diseño de este estudio no sigue el paradigma clásico de la manipulación neonatal, resulta interesante ya que se observa claramente que los diferentes estímulos presentes durante la vida temprana, pueden influenciar de manera importante la aparición de diferencias individuales.

En otro estudio más reciente (Gazzano et al., 2008) también realizado con perros, la manipulación se llevó cabo separando a los cachorros 5 minutos al día desde el 3er día de vida hasta el final del período neonatal (día 21 de vida). Cuando los cachorros fueron testeados a las 8 semanas de edad, se encontraron diferencias entre cachorros manipulados y no manipulados. Así, los cachorros manipulados mostraron menos signos de estrés, siendo este efecto más marcado en animales procedentes de criaderos que en comparación con animales criados en un ámbito familiar.

La manipulación neonatal también afecta la síntesis de neurotransmisores. Así, por ejemplo, la manipulación neonatal incrementa los niveles de serotonina cerebral (Papaioannou et al., 2002). Este hecho es muy importante si consideramos que muchos problemas de conducta en perros se relacionan, al menos en parte, con una disminución en la actividad de dicho neurotransmisor (Reisner et al., 1996; Wright et al., 2012). De hecho, varios fármacos utilizados en etología clínica veterinaria actúan aumentando la actividad serotoninérgica central (Manteca, 2003).

Partiendo de la premisa de que uno de los efectos clave de la manipulación neonatal es precisamente la manipulación de la conducta maternal que logra aumentar la conducta de lamido sobre las crías y así la estimulación táctil recibida por los cachorros, podríamos deducir que la diferencias individuales en conducta maternal también jugarían un papel importante en las diferencias individuales en la capacidad de adaptación a situaciones de estrés de las crías. De hecho, en roedores de laboratorio se ha demostrado la existencia de diferencias en conducta maternal que resultan en diferentes niveles de estimulación táctil provista a los cachorros. Liu et al., (1997) observaron que los cachorros cuyas madres presentaban una alta frecuencia de conducta de lamido se adaptaban mejor a situaciones de estrés cuando eran adultos que los cachorros control. Cuando llegan a una edad adulta, además, las crías de las madres que exhiben una mayor frecuencia de conducta de lamido, muestran una

reducida concentración de ACTH y corticosterona plasmáticas en respuesta al estrés agudo y un incremento de la sensibilidad de los mecanismos de retroalimentación (Liu et al., 1997). También se observan diferencias en conducta, así, las crías de madres que presentan una alta frecuencia de conducta de lamido, muestran una respuesta de sobresalto menor, un aumento de la conducta exploratoria durante el OFT y una menor latencia para comer en un ambiente nuevo (Caldji et al., 1998).

El rol de la perra sobre el comportamiento de las crías también ha sido descrito en algunos trabajos (Scott y Fuller, 1965; Wilson y Sundgren, 1998; Stranbderg et al., 2005). Piñol et al., (2005) observaron un incremento de la conducta de lamido de las perras luego de la separación de sus cachorros. Sin embargo, el efecto sobre la conducta de los cachorros no fue explorado y para nuestro conocimiento no existe ningún estudio en perro de esas características.

2.2.3. Período de socialización

El período de socialización del perro, se extiende desde el final del período neonatal -3 semanas de vida- hasta las 12 semanas de vida (Scott, 1958). Los aprendizajes que adquiere el cachorro durante este período, pueden tener un efecto duradero sobre su comportamiento (Manteca, 2003) y pueden ejercer un efecto muy importante en las diferencias individuales en conducta. Existen diversos estudios que ponen en evidencia el efecto del ambiente y aprendizaje durante el período de socialización sobre la conducta del perro. El contacto con la madre durante esta etapa, por ejemplo, parece jugar un rol importante para el desarrollo conductual de los cachorros en esta etapa (Wilsson, 1984). Así, por ejemplo, el destete precoz puede tener efectos negativos sobre la conducta futura de individuo. Este hecho queda reflejado en el trabajo de Pieratoni y Verga (2007) en el que se observó que los cachorros que eran separados de la madre a una edad temprana (30-45 días) tenían mayores probabilidades de mostrar conductas relacionadas con el miedo y la ansiedad que los cachorros que permanecían con la madre hasta los dos meses de edad.

Durante este período, además, es cuando se produce el proceso de socialización primaria (Serpell y Jagoe, 1995). Los cachorros que no tengan contacto con individuos de su propia especie estarán más predispuestos a presentar problemas para comunicarse con otros perros e incluso podrían mostrar miedo y/o agresividad hacia los mismos. Asimismo, el escaso contacto con otras especies animales o personas, predispone a presentar problemas de miedo o agresividad frente a esas especies a las que no fueron expuestos (Beaver, 2009). Es decir que las experiencias sociales durante este período no sólo definen la especie a la cual pertenece el animal sino que determinan a los futuros compañeros sociales, (Serpell y Jagoe, 1995). En el caso particular de la socialización con personas, el proceso de reconocimiento es visual, es decir que para un cachorro un niño no necesariamente es lo mismo que una persona adulta (Manteca, 2003). En un estudio retrospectivo, se observó que perros adultos que no habían tenido contacto con niños durante el período de socialización mostraban un mayor riesgo a mostrar agresividad y excitabilidad ante la presencia de un niño que aquéllos que sí habían tenido contacto (Arai et al., 2011).

Además del proceso de socialización, durante este período se produce un proceso de habituación a estímulos. Cuanto mayor sea la variedad de estímulos a los cuáles sea expuesto el animal, menor será el grado de neofobia que presentará cuando sea adulto. Los cachorros criados en ambientes pobre a estímulos además, son propensos a presentar miedo a estímulos y en casos más severos inhibición general de la conducta (Beaver, 2009).

3. Evaluación de las diferencias individuales en conducta y estrés

3.1. Evaluación de la conducta

Un primer paso para estudiar las diferencias individuales en comportamiento es lógicamente detectarlas para luego intentar identificar los factores potenciales que pueden determinarlas.

Las diferencias individuales en comportamiento pueden evaluarse por medio de diferentes métodos, cada uno de las cuales tiene ventajas y desventajas. Los métodos para evaluar el comportamiento deberían cumplir ciertos requisitos que no siempre se cumplen: deben ser fiables y válidos. La fiabilidad es un prerrequisito para la validez y se refiere a la ausencia de errores de medida, o dicho de otro modo, al grado de consistencia y estabilidad de las puntuaciones obtenidas a lo largo de sucesivos procesos de medición con un mismo instrumento. Las medidas de fiabilidad incluyen la consistencia dentro de mismo observador, entre observadores, dentro del mismo perro (test-retest) y dentro de los componentes de medición designados a evaluar la misma conducta (consistencia interna) (Taylor y Mills, 2006). Una de las claves de los métodos de evaluación del comportamiento es la demostración de la fiabilidad del test cuando se vuelve a repetir (test-retest).

La validez indica con qué precisión un instrumento mide lo que se supone que debe medir (Jones y Gosling, 2005). Los métodos de evaluación del comportamiento tienen que asegurar que en realidad están evaluando el rasgo de comportamiento de interés (ej. miedo). La probabilidad de alcanzar ese objetivo incrementa utilizando contextos limitados como en los test de conducta (Taylor y Mills, 2006).

Mendl y Harcourt (2000) proponen las siguientes formas de evaluación de las diferencias individuales en conducta: a. Registro de conductas en una situación no controlada; b. Registro de conductas en una situación controlada y estructurada (tests); c. Puntuación por parte de observadores; d. Reporte por parte de los propietarios de los individuos.

a. Registro de conductas en una situación no controlada. Este método constituye una manera fiable de distinguir diferencias individuales en términos de frecuencia, duración y patrones de conducta. Sin embargo, el hecho de que se haga de una manera no controlada, no permite predecir la conducta del animal en un contexto diferente. Además, la evaluación de la consistencia de la conducta es complicada porque pueden no darse las mismas condiciones en cada evaluación. Un ejemplo de este tipo de evaluación es el trabajo de Goddard y Beilharz, (1986). En el mismo, los

investigadores evaluaron ciertas conductas de perros que se presentaban durante el paseo, cuando se les permitía correr sin correa y en la casa.

b. Registro de conductas en una situación controlada y estructurada (tests). Los test se realizan en condiciones experimentales estandarizadas donde se provoca la aparición de una respuesta frente a estímulos específicos y los resultados son estadísticamente comparados con los de otros individuos puestos en la misma situación (Hsu y Serpell, 2001; Jones y Gosling, 2005). Los test constituyen el método más frecuentemente utilizados para evaluar diferencias individuales en conducta tanto en perros (Wilson y Sundgren, 1997; Slabbert y Odendaal, 1999; Svartberg y Forkman, 2002) como en otras especies (Forkman et al., 2007). Debido a las condiciones estandarizadas en que se realizan, en principio estos test representarían el método más objetivo de evaluar la conducta.

El test de Open Field (OFT) fue diseñado por primera vez para evaluar la respuesta de miedo en ratas (Hall, 1934). Este test consiste en colocar al animal en un espacio desconocidos y cerrado de manera que no pueda escapar (Walsh y Cummins, 1976) y evalúa ciertas conductas realizadas por el mismo en un periodo de tiempo determinado. Las respuestas de miedo se producirían principalmente como consecuencia de colocar al animal en un ambiente desconocido y al aislamiento social (Walsh y Cummins, 1976). El OFT ha sido ampliamente utilizado en varias especies domésticas como el vacuno (De Pasillé et al., 1995), cerdos (Donald et al., 2011), ovejas (Pedernera-Romano et al., 2010), gatos (Marchei et al., 2009) y perros (Head et al., 1997). Uno de los inconvenientes del OFT, es que la respuesta mostrada por el animal durante el test puede estar influenciada por muchos factores independientes al grado de miedo como la estrategia de adaptación del individuo, la edad, el sexo, capacidad aeróbica, etc. (DePasillé et al., 1995). Landsberg et al., (2009) por ejemplo, observaron una menor distancia total recorrida durante el OFT en perros miedosos en comparación con perros no miedosos, mientras que Araujo et al., (2010) no vieron afectada esta variable como resultado del miedo. Esto significa que probablemente haya otros factores independientes al miedo que afectan dicha conducta. La

interpretación de las conductas mostradas por el animal también suele resultar difícil. Así, por ejemplo, el nivel de actividad en terneros durante el OFT fue interpretada por Warnick et al., (1977) como indicadora del grado de nerviosismo y como indicadora del grado de motivación por realizar la conducta de locomoción por Dantzer et al., (1983).

Por todo lo dicho, se ha sugerido la implementación de métodos más sofisticados para analizar de una manera multidimensional las respuestas durante el OFT, como el análisis factorial o de componentes principales, en vez de guiarse sólo por conductas aisladas (Ramos y Mormède, 1998). Estos métodos tienen la ventaja de que permiten comprender las relaciones entre diferentes rasgos de conducta (Manteca y Deag, 1993).

c. Puntuación por parte de observadores. Este método consiste en que dos o más observadores, que conozcan bien a los animales, puntúen a los individuos de manera independiente basándose en categorías conductuales definidas (Mendl y Harcourt, 2000). La fiabilidad de estas puntuaciones puede ser determinada calculando las correlaciones entre las puntuaciones adjudicadas por cada individuo y por la comparación con métodos de observación de patrones relacionados con dichas categorías (Feaver et al., 1986). En un estudio realizado en gatos, se demostró la fiabilidad y validez de este método para evaluar diferencias individuales en ciertas categorías conductuales del gato, por ejemplo: agresividad, curiosidad, excitabilidad, miedo a gatos, miedo a personas, etc. (Feaver et al., 1986). Sin embargo, existe cierto riesgo de subjetividad relacionado con el papel activo que tienen los observadores. Otra desventaja es que desde el punto de vista práctico tiene la limitación de que se necesita que los observadores conozcan bien a los individuos que participan en el estudio, lo que puede llevar cierto tiempo (semanas o meses) (Mendl y Harcourt, 2000).

d. Reporte por parte de los propietarios de los individuos. Este método se basa en la recolección de información de la conducta de ciertos individuos a partir de un

informante (Jones y Gosling, 2005) que suele ser el propietario del perro. Así, se realizan preguntas en las que el dueño tiene que responder si su perro realiza o no ciertas conductas o con qué frecuencia las realiza (ej. gruñir cuando le quitan el plato de comida) (Podberscek y Serpell, 1996; Hsu y Serpell, 2003). Se podría decir que este método es hasta cierto punto subjetivo, sin embargo, Block, (1961) demostró que cuando las puntuaciones eran agrupadas, este método dejaba de ser subjetivo. Además, tiene la ventaja de que se puede evaluar la conducta del animal en su ambiente y con las personas con las que convive (ej. en su casa con su propietario) y esto es especialmente importante en animales de compañía como el perro. Un ejemplo de este tipo de evaluación es el cuestionario C-BARQ (Canine Behavioral Assessment and Research Questionnaire) diseñado por Hsu y Serpell, (2003). El mismo consiste en 101 preguntas que el dueño tiene que contestar acerca del comportamiento del perro. A partir de aquí se extrajeron 13 rasgos de conducta por medio de un análisis factorial. La consistencia interna de cada factor fue examinada mediante el coeficiente Alfa de Cronbach, obteniendo resultados satisfactorios en todos los factores excepto en uno. Por otro lado, la validez de los factores extraídos se examinó comparando las respuestas obtenidas en 203 cuestionarios con el diagnóstico realizado por un especialista en Etología Clínica (Hsu y Serpell, 2003).

3.2. Evaluación de la respuesta fisiológica de estrés.

3.2.1. Evaluación del eje HPA

La interpretación de la respuesta del eje HPA está lejos de ser simple (Momèrde et al., 2007). Los motivos que dificultan la interpretación de la respuesta de estrés son los siguientes: en primer lugar, dichos sistemas están involucrados en la homeostasis general del organismo, no sólo en la respuesta de estrés. Así, la activación de alguno de estos sistemas no necesariamente corresponde a una respuesta frente a un estímulo estresante. Por ejemplo, existe un incremento de los niveles de cortisol inducido por las comidas (Momèrde et al., 2007). En segundo lugar, la duración del estímulo estresante puede también dificultar la interpretación. Por ejemplo, si el

estímulo estresante se mantiene en el tiempo, los niveles circulantes de glucocorticoides vuelven a sus niveles basales, incluso si se puede detectar una sostenida activación del eje HPA mediante tests específicos. Por otro lado, el cortisol, tiene un ritmo circadiano que puede resultar en variaciones en su concentración a lo largo del día. Debido a esto, se recomienda tomar las muestras siempre a la misma hora. Las mayores concentraciones de cortisol plasmático en el perro se dan por la mañana (Beerda et al., 1999). En perros, los la concentración de cortisol puede evaluarse en plasma, saliva, orina, heces y pelo. Cada método tiene ventajas y desventajas.

a. Cortisol en plasma.

La determinación de cortisol plasma para evaluar el bienestar animal y la respuesta de estrés ha sido utilizada en prácticamente todas las especies domésticas (Mormerde et al., 2007), incluido el perro (Hennessy et al., 1997; Hennessy et al., 1998; Hennessy et al., 2001; Steiss et al., 2007).

Uno de los principales inconvenientes de la obtención de cortisol en sangre es su carácter invasivo (Dreschel and Granger, 2009). Además, luego de un evento que el animal percibe como estresante, la elevación del cortisol en sangre se produce rápidamente y se debe tener en cuenta, que la elevación del cortisol es sensible también a muchos factores ambientales, por ejemplo, el simple hecho de coger al animal y sujetarlo puede llevar a un incremento de esta hormona, pudiendo confundir los resultados. Es decir que podríamos confundir entre la elevación se da por el potencial estímulo estresante que queremos evaluar y el hecho de sujetar al animal. Para evitar que este inconveniente se debería tomar la muestra entre los 2 y 3 minutos de haber cogido al animal o habituar previamente al animal a la maniobra (Mòrmede et al., 2007) ya que el incremento de la concentración sanguínea de cortisol asociada a la maniobra de recolección generalmente ocurre a los 3 minutos desde el comienzo del manejo (Tuber et al., 1996).

b. Cortisol en orina.

La evaluación de cortisol en orina tiene la ventaja de puede ser un método no invasivo. Los productos de excreción se acumulan a lo largo de varias horas, lo que permite obtener una medición integral de la producción de cortisol en un período de tiempo determinado (Mórmede et al., 2007). Este método, sin embargo, no es adecuado para evaluar respuestas de estrés agudo. Los niveles de hormonas se expresan como radios hormona/creatinina de manera de poder evaluar las diferencias en la producción de orina ya que la creatinina es secretada a un ritmo constante (Mórmede et al., 2007).

El método de medición de cortisol en orina para evaluar estrés ha sido validado por Beerda et al. (1996). Ejemplos de medición de cortisol en orina en perros pueden encontrarse en los trabajos realizados por Beerda et al., (1999) y Blackwell et al.,(2010).

c. Cortisol en heces.

La evaluación de cortisol en heces resulta también un método no invasivo para evaluar el estrés y el bienestar animal (Mòmerde et al., 2007). Otra ventaja de la evaluación del cortisol en heces es que, a diferencia de las muestras de sangre por ejemplo, las muestras fecales se encuentran menos afectadas por fluctuaciones episódicas o diurnas (De Palma et al., 2005). Este método, sin embargo, no resulta apropiado para evaluar respuestas de estrés agudo. Schatz y Palme, (2005) comprobaron que la medición de cortisol en heces era un método efectivo para evaluar la actividad adrenocortical.

d. Cortisol en pelo.

También tiene la ventaja de no ser invasivo. El análisis del cortisol en pelo es particularmente útil en estudios de bienestar animal y estrés crónico que requieren una monitorización de la función adrenal durante períodos prolongados (Accorsi et al., 2008). Tomar una muestra de pelo es relativamente sencillo y es fácil de conservar.

Dos muestras (rasurado y pelo que vuelve a crecer) son suficientes para la evaluación del perfil hormonal. Una desventaja es que no provee información de cambios que suceden en períodos cortos de tiempo (Koren et al., 2002).

e. Cortisol en saliva.

Una de las principales ventajas de la evaluación de cortisol en saliva, es el carácter poco invasivo de su recolección. La concentración de cortisol en saliva ha demostrado estar correlacionada con la concentración de cortisol en sangre (Beerda et al., 1996). A pesar de que se necesita sujetar al animal, la extracción de saliva no constituye un método invasivo como la obtención de muestra de sangre. Además, se ha observado que la extracción de saliva puede llevar hasta 4 minutos sin que haya un efecto de la sujeción sobre las concentraciones de cortisol (Kobelt et al., 2003). Las muestras de saliva son relativamente fáciles de recolectar sin mucho entrenamiento y se pueden recolectar volúmenes suficientes en la mayoría de los perros (Dreschel and Granger, 2009) ya que la mayoría de los kits que analizan cortisol en saliva utilizan sólo 25 µl de saliva para cada determinación. El rango de sensibilidad de los tests es de 0.007 a 1.8 µg/ dl y los coeficientes de variación promedio dentro y entre ensayos es menor del 10 % y 15 % respectivamente (Dreschel and Granger, 2009). El cortisol salival ha sido utilizado como medida de estrés en perros en numerosos estudios (Beerda et al., 1996; Beerda et al., 1998; Beerda et al., 1999; Dreschel and Granger, 2005; Horváth et al., 2007).

3.2.2. Evaluación del sistema serotoninérgico

La relación entre el sistema nervioso serotoninérgico y la hiperactividad del eje HPA ha sido reconocida (Reimold et al., 2011). Durante el estrés crónico, por ejemplo, se producen cambios en el sistema serotoninérgico que consisten en una disminución de la densidad de los receptores $5HT_{1A}$ (Leonard, 2005). Estos cambios podrían ser relevantes para comprender la relación entre el estrés y patologías relacionadas con la ansiedad.

La concentración de serotonina puede determinarse en el líquido cefalorraquídeo (LCR) (Reisner et al., 1996). Sin embargo, este método resulta muy invasivo y, por lo tanto, poco práctico para llevar a cabo de manera habitual. En personas, se ha observado, que existe una correlación entre las concentraciones de serotonina del LCR y la serotonina sérica (Sarrias et al., 1990), lo cual permite la posibilidad de evaluar la concentración de este neurotransmisor de una manera menos invasiva.

En perros, se ha observado una menor concentración de del principal metabolito de la sertotonina, el ácido 5 hidroxiindolacético (5-HIAA) en el líquido cefalorraquídeo (LCR) de perros agresivos en comparación con perros no agresivos (Reisner et al., 1996). Asimismo, otros estudios en perros, han evaluado la concentración serotonina en suero y también han observado que la misma era menor en perros agresivos (Cakiroglu et al., 2007; Rosado et al., 2010). En el estudio de Rosado et al., (2010), se observó que además de una menor concentración de serotonina sérica, los perros agresivos tenían una mayor concentración de cortisol plasmático, sugiriendo una posible conexión entre el sistema serotoninérgico y el eje HPA, al igual de lo que se ha comprobado en roedores de laboratorio y humanos (Leonard, 2005).

3.2.3. Evaluación del SNA. Hipertermina inducida por estrés (HIE).

La respuesta del SNA frente a una situación de estrés puede abordarse evaluando catecolaminas, presión arterial, frecuencia cardiaca, frecuencia respiratoria e hipertermia inducida por estrés.

En el caso particular de la HIE, este término hace referencia al incremento transitorio de la temperatura corporal que se produce en respuesta a una situación de estrés y que es comparable en las diferentes especies animales (Vinkers et al., 2008). Los fármacos ansiolíticos, incluyendo las benzodiacepinas, bloquean la respuesta de la HIE (Vinkers et al., 2009). Esta elevación de la temperatura suele ser de corta duración, aproximadamente 15 minutos y la elevación suele ser de entre 1.0-1.5 ºC. La HIE se presenta en prácticamente todas las especies mamíferas en las que ha sido testeada (Bouwknecht et al., 2007). En el caso particular del perro, se observó HIE como parte

de una respuesta autonómica asociada a un estímulo de miedo condicionado (Ogata et al., 2006a).

4. Diferencias individuales en conducta agresiva

El estudio de las diferencias individuales en conducta agresiva es de especial interés por varios motivos. En primer lugar, los problemas de agresividad canina representan un riesgo considerable para la salud pública de las personas (Wright, 1990; Guy et al., 2001; Rosado et al., 2009). Estudios epidemiológicos realizados en Estados Unidos han demostrado que en ese país, 1 a 4,5 millones de personas son mordidas por perros cada año, de las cuales aproximadamente 700000 necesitan atención médica (Sacks et al., 1996). La agresividad canina también puede ser un problema cuando se dirige hacia otros perros u otros animales. Los problemas de agresividad canina, además, son los problemas más frecuentemente consultados en los servicios de Etología Clínica (Overall, 1997; Fatjó et al., 2006; Fatjó et al., 2007). Fatjó et al., (2007), por ejemplo, observaron que los problemas de agresividad representaban el 52, 28% de todos los casos de perros que acudían a un servicio de Etología Clínica. Por último, la agresividad también puede afectar el bienestar del propio individuo que la manifiesta. Así, la agresividad de tipo afectiva, que es la que se observa con mayor frecuencia, resulta desagradable para el animal e involucra una respuesta de estrés (Kurk et al., 2004). Además, los perros con agresividad pueden ser abandonados (Salman et al., 1998; Salman et al., 2000) e incluso eutanasiados sólo por el hecho de ser agresivos (Overall, 1997).

Existen diferentes aproximaciones a la hora de clasificar los problemas de agresividad. La clasificación puede ser biológica, que depende básicamente de los centros nerviosos implicados en su control, o clínica que depende más bien del contexto en el que se producen los eventos agresivos. En cuanto a la clasificación biológica, se distinguen 3 tipos de agresividad: ofensiva, defensiva y depredadora. Cada uno de estos tipos está controlado por estructuras diferentes del SNC (Sistema Nervioso Central) (Siegel y

Shaikh, 1997). La postura que caracteriza a cada uno de estos tipos de agresividad es diferente. Una postura típicamente ofensiva se presenta con el cuerpo erguido, las extremidades extendidas, las orejas erguidas y hacia adelante, la cola levantada, la mirada fija y los labios retraídos mostrando sólo los incisivos y los caninos (Fox y Bekoff, 1975; Ferris et al, 1997; Blanchard y Blanchard, 2006). La postura defensiva, en cambio, se caracteriza porque el animal mantiene el cuerpo agachado, con las orejas hacia atrás, la cola baja, el pelo del lomo erizado y los labios retraídos mostrando también los molares (Fox y Cohen, 1977; Houpt, 1998). Por último, la agresividad depredadora comprende todas o algunas de las diferentes partes de la secuencia de depredación como acechar, perseguir, capturar y matar a la presa (Beaver, 1999). Estos tres tipos de agresividad, además, se diferencian según exista o no estimulación simpática (Blanchard y Blanchard, 2006). Así, las agresividades de tipo defensiva y ofensiva van acompañadas de una marcada actividad autonómica que involucra una fuerte activación simpática por lo que se las incluye dentro del grupo de agresividad afectiva. La agresividad depredadora, por otro lado, no se acompañan de activación simpática por lo que se la llama agresividad de tipo no afectiva (Manteca, 2003).

En cuanto a la clasificación clínica, la misma depende del contexto en el que se produce la agresividad y se basa en la posible motivación del perro para mostrar agresividad. Desafortunadamente no existe un consenso entre los especialistas en Medicina del comportamiento de animales de compañía y cada uno ha propuesto una clasificación diferente (Overall, 1997; Beaver, 1999; Landsberg, 2003; Leuscher y Reisner, 2008). Otros autores incluso prefieren simplemente describir objetivamente el fenómeno que están investigando, sin necesariamente otorgar un nombre específico al problema (Mills, 2006). Una manera práctica de clasificar los problemas de agresividad es teniendo en cuenta tres parámetros: el blanco hacia el cual va dirigido el ataque (por ejemplo, persona de la familia, persona desconocida, perros de la familia, etc.), el contexto en el que se produce la agresividad (por ejemplo, competencia por un recurso, acercamiento, invasión del territorio, etc.) y la postura del perro (por ejemplo, ofensiva, defensiva o ambivalente). De esta manera se puede intentar abordar la motivación del perro para mostrar agresividad. Se debe tener en cuenta, sin embargo,

que en muchas ocasiones los perros presentan varias motivaciones al mismo tiempo (Bowen y Heath, 2005) por lo que el abordaje no siempre es sencillo.

Diversos factores han sido involucrados en las diferencias en conducta agresiva. Tal como lo vimos más arriba, tanto la genética como las experiencias de las etapas tempranas de la vida pueden ejercer un rol importante en la predisposición a manifestar agresividad (Mertens, 2002). La interacción de esos factores predisponentes con factores ambientales y de manejo de los propietarios, juegan un rol muy importante en la aparición de estas conductas.

Ciertos factores ambientales, especialmente aquéllos relacionados con el manejo del propietario han sido asociados a la conducta agresiva en perros. Así, por ejemplo, se ha observado una correlación entre el manejo inconsistente por parte de dueño y la conducta agresiva (Cullinan et al., 2004; Arhant et al., 2010). Cuando hablamos de manejo inconsistente, nos referimos a una falta de comunicación clara entre el dueño y su perro. Esto podría suceder, por ejemplo, cuando una misma conducta es castigada en determinadas ocasiones y reforzada en otras. Este tipo de interacciones crean un ambiente impredecible e incontrolable para el individuo, y pueden generar frustración, ansiedad, estrés y agresividad en el animal (Leuscher y Reisner, 2008).

Otro factor relacionado con la agresividad canina es la utilización de castigos. Arhant et al., 2010 por ejemplo, observaron una relación entre la utilización frecuente de castigos y un incremento de la ansiedad y agresividad en perros. Por su parte, en el trabajo de Hiby et al. (2004) se observaron un incremento de conductas problemáticas en perros en los cuáles utilizaban castigos. En otro estudio, se observó que los dueños que utilizaban castigos tenían perros con un score medio de agresividad mayor que los dueños que utilizaban sólo refuerzos como métodos de entrenamiento (Blackwell et al., 2008). El uso de castigos podría incrementar conductas asociadas con la ansiedad.

Otros factores relacionados con el manejo del propietario, como el hecho de permitirle dormir cerca del mismo, el motivo por el cual se ha decidido adoptar un perro y el hecho de tener un perro por primera vez, han sido asociados con una mayor prevalencia de conductas agresivas (Jagoe y Serpell, 1996). La procedencia de perro también parece tener un efecto importante sobre la conducta agresiva. Serpell y Jagoe (1995) observaron que los perros que procedían de tiendas de mascotas presentaban problemas de agresividad por dominancia y miedo social con más frecuencia que los que procedían de otros orígenes. La relación entre estos dos factores se explicaría por la escasa socialización que probablemente tengan los perros que permanecen en tiendas de mascotas.

La falta de ejercicio físico parece jugar un rol importante en la aparición de la conducta agresiva. El ejercicio físico se asocia con un incremento en los niveles de serotonina en humanos y roedores de laboratorio (Chaouloff, 1997). Para nuestro conocimiento, no existen publicaciones en perros donde se constate esta asociación directa entre niveles de serotonina y ejercicio; sin embargo, sí existen estudios donde se observa una relación entre altos niveles de ejercicio físico y una menor presentación de la conducta agresiva en perros (Jagoe y Serpell, 1996). El hecho de que el ejercicio pueda aumentar los niveles de serotonina cerebral tiene una importancia relevante en los problemas de agresividad canina ya que, como se ha comentado más arriba, se ha observado una relación entre este neurotransmisor y la agresividad (Reisner et al., 1996; Cakiroglu et al., 2007).

Las hormonas sexuales también han demostrado influenciar las diferencias individuales en conducta agresiva. Así, se ha observado una mayor incidencia de agresividad en machos que en hembras (Borchelt, 1983; Reisner et al., 2005; Fatjó et al., 2007; Amat et al., 2009), especialmente en la agresividad de tipo ofensiva (Nelson, 2005). En perras agresivas, sin embargo, la castración puede aumentar la intensidad de esta conducta (O'Farrell y Peachey, 1990). Este hecho, parece estar asociado a la

supresión del efecto inhibitorio que ejerce la progesterona sobre la conducta agresiva (Hart y Eckstein, 1997).

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OBJETIVOS

1. Caracterizar las diferencias individuales en la conducta agresiva del perro doméstico mediante el cuestionario C-BARQ®.

2. Valorar el efecto de la manipulación neonatal, la edad, y la camada sobre las diferencias individuales en la respuesta fisiológica de estrés en cachorros.

3. Identificar diferencias individuales en la respuesta conductual frente a la novedad y el aislamiento y evaluar el efecto de la manipulación neonatal y de la camada sobre dicha respuesta.

4. Evaluar las características de la agresividad del perro hacia personas de la familia y los factores causales que podrían contribuir a las diferencias individuales en la expresión de dicha conducta.

CAPÍTULO I

Assessing aggressive behavior in dogs through the C-BARQ©

Susana Le Brech, Marta Amat, Tomás Camps, Valentina Mariotti, Déborah Temple and Xavier Manteca

Abstract

Canine aggression is the most frequent behavioral problem in dogs. The aim of this study was to assess behavioral traits related to aggression in 82 dogs presented at the Animal Behavior Service of the School of Veterinary Science in Barcelona, Spain, through the Canine Behavioral Assessment and Research Questionnaire (C-BARQ©). Questionnaires were completed by the owners prior to the beginning of the first clinical appointment. Positive correlations were found between the traits owner directed aggression and attachment or attention seeking behavior (r_S=0.44, P<0.001) and excitability (r_S=0.33, P<0.01). Chasing was positively correlated to dog directed aggression (r_S=0.51, P<0.001) and stranger directed aggression (r_S=0.31, P<0.01). Different hypothesis to explain such correlations are discussed. Additional research on larger sample size is needed.

Introduction

Aggression is the most common behavioral problem in dogs (Bamberger and Houpt, 2006; Fatjó et al., 2007). Apart from its frequency, this problem may compromise animal welfare because, as other behavioral problems, it constitutes an important cause of abandonment (Salman et al., 1998; Salman et al., 2000) and euthanasia (Overall, 1997). In addition, there is evidence that aggressive behavior may involve a stress response (Menno et al., 2004) challenging the physiological and psychological homeostasis. Furthermore, canine aggression represents a serious public concern as it can cause physical and emotional damage to people and has very high hidden costs to communities (Beaver et al., 2001).

Individual differences in the way the animals responds to the environment have been reported in many domestics animals (eg. Lyons et al., 1988; Jensen et al., 1995; Mülleder et al., 2003). The concept of temperament emerges to explain these individual differences observed when testing animals and it considers that such differences are present at an early age and are relatively consistent over time (Weiss and Greenberg, 1996; Diederich and Giffroy, 2006; Svartberg et al., 2005). Temperament can be influenced by various factors such as genetics (Scott and Fuller, 1965), prenatal environment (Griffin et al., 2003) and early experiences (Caldji et al., 1998; Clinton et al, 2007). In the particular case of canine aggression, some environmental factors such as the characteristics of the owners and management have been shown to affect the expression of aggressive behavior (Podberscek and Serpell, 1997a). It was observed, for instance, that owners of high aggressive dogs were more likely to be tense, emotionally less stable, shy and undisciplined than owners of low aggressive dogs (Podberscek and Serpell, 1997b). Also, dogs belonging to first time owners were found to show dominance related aggression more frequently than dogs belonging to experienced owners (Jagoe and Serpell, 1996). In relation to the management, obedience training and sleeping close to the owner were found to be

associated to a reduced and an increased prevalence of competitive aggression respectively (Jagoe and Serpell, 1996). The use of punishment was also associated with an increase aggression in dogs (Arhant et al., 2010).

Temperament traits can be assessed by means of temperament tests or questionnaires (Jones and Gosling, 2005). When measuring behavior the method of assessment should meet some specific requirements. In the first place, it has to be standardized with the only variable measured being the animal tested, controlling all potential sources of variability. Second, it must be repeatable, i.e. free from random errors. Also, it has to be valid, measuring what the investigator actually wishes to measure and providing the information that is relevant to the questions being asked (Martin and Beateson, 2007).

Temperament tests evaluate the dog's reaction towards specific standardized situations (Jones and Gosling, 2005; Taylor and Mills, 2006), each test having different objectives. In theory, the tests are more objective than the questionnaires, as the behavior of the animal can be directly measured, but they have some inconveniences. For example, it may not be easy to elicit the behavior that has to be assessed (Christensen et al., 2007) and frequently the behavior of the dog during the test is not representative of its behavior in non-test situations (Van den Berg et al., 2003). In the particular case of the tests that evaluate aggressive behavior, precautions are needed to ensure the safety of the person that performs the test (Netto and Planta, 1997).

Questionnaires, however, can be completed by the owner who describes the dog’s responses to specific situations and events. One of the main advantages of the questionnaires is that they can evaluate many behavioral reactions that can not be measured by temperament tests (Hsu and Serpell, 2003). Additionally, the behavior of the dog is described in its natural environment. Although questionnaires are often

seen as subjective, some authors suggest that the information obtained is reliable (Jones and Gosling, 2005).

Hsu and Serpell (2003) developed a questionnaire (Canine Behavioral Assessment and Research Questionnaire – C-BARQ©) that consists of 101 items designed to measure the behavior and temperament of pet dogs. Using exploratory factor analysis, 11 factors were initially extracted: stranger-directed aggression (SDA), owner- directed aggression (ODA), dog-directed fear or aggression (DDFA), stranger-directed fear (SDF), non-social fear (NSF), separation related behaviors (SRB), attachment or attention seeking behavior (AAS), trainability (TRAIN), chasing (CHAS), excitability (EX) and touch sensitivity (TS). The internal consistency of these factors was examined by calculating the value of Cronbach's α. All factors, except touch sensitivity, had adequate α values (Hsu and Serpell, 2003). Following initial publication of the questionnaire, some items related to aggression and fear towards unknown people and touch sensitivity were added or modified to improve the reliability of the relevant subscales. Also, some items regarding fearful or aggressive responses when interacting with other dogs were incorporated in order to be able to segregate the “dog -directed fear and aggression” subscale into two subscales, “dog-directed fear” and “dog-directed aggression” and to include a new subscale “familiar dog rivalry” (DR). In addition, a new "energy" (ENE) subscale was included.

The aim of this study was to identify behavioral characteristics related to aggression through a correlation analysis of the C-BARQ’s factors.

Materials and Methods

Sample and questionnaire

Behavioral data was obtained from all dogs presented at the Animal Behavioral Service of the School of Veterinary Science in Barcelona, Spain, between 2008 and 2009 and using the C-BARQ translated into Spanish. Except for items 75, 76, 77 and 92 and 93 that are necessary to calculate the factors CHAS and ENE respectively, questions of miscellaneous section were excluded from the study to reduce the time needed to fill the questionnaire. The factor DR was not calculated because the number of dogs that shared a home with another dog was too small. The subscale DDFA was segregated into DDF and DDA. Additional information from the dog (breed, age, gender and neuter/ spay status) was also collected. The questionnaires were completed by the owners prior to the beginning of the first clinical appointment in order to avoid any bias. The owners had approximately 20 minutes to fill out the questionnaire and once it was completed it was returned directly to the researchers. Questionnaires with incomplete answers and from dogs that had already been at a behavioral consultation were eliminated from the study.

The owners had to indicate how their dogs have responded in the recent past (i.e. the latest 1-2 months) to a variety of common events and stimuli, using a 5 point rating. Depending on the question, the behavior was evaluated in terms of frequency (0 = never, 1 = seldom, 2 = sometimes, 3 = usually and 4 = always) or intensity (0 = no signs of the behavior, 1 – 3 = mild to moderate signs of the behavior, and 4 = severe signs of the behavior). In the heading of each section there was a brief explanation describing the behavioral signs that owners could use as a guide to score the dogs.

Statistical analysis

Behavioral scores were calculated as the average of the C-BARQ items that comprise each factor (e.g. score for owner directed aggression: score for questions 9 + 13 + 14 + 17 + 19 + 25 + 30 + 31/ 8).

Because the data did not follow a normal distribution and could not be corrected with data transformation, a non-parametric test was used. In order to evaluate the possible associations between factors, Spearman rank correlation coefficients were used. The statistical package SAS (9.1, SAS Institute Inc., Cary, NC, USA) was used to analyze the data.

Results

Descriptive analysis

From the total of questionnaires obtained (n= 109), 82 (75 %) were suitable for analysis. Thirty four dogs (41 %) were females (68 % of which were intact) and 48 (59 %) were males (73 % of which were intact). The mean age of the animals was 3.2 years-old (SD= 2.56). The breeds of the dogs that participated in this study are presented in Table1. According to the behavioral diagnosis made by the specialists during the consultation, 27 (32.9%) dogs showed aggression towards owners, 22 (26.8%) showed aggression towards unknown people and 29 (35.3%) dogs showed dog-directed aggression.

Table 1. Breeds of dogs that participated in the study.

Breed	**N**
Alaskan Malamute	1
Argentine Dogo	1
Beagle	1
Bobtail	1
Border Collie	1
Boxer	4
Bull Terrier	1
Catalan Sheepdog	3
Chihuahua	1
Chow Chow	2
Doberman Pinscher	2
English Bulldog	1
English Cocker Spaniel	2
French Bulldog	5
German Shepherd	3
Golden Retriever	2
Jack Russell Terrier	1
Labrador Retriever	5
Maltese	1
Miniature Schnauzer	2
Neapolitan Mastiff	1
Parson Russell Terrier	1
Pekingese	1
Pit Bull Terrier	1
Poodle	3
Pointer	1
Shetland Sheepdog	1
Spanish Water Dog	2
Terranova	1
Vizla	1
West Highland White Terrier	3
Crossbreed	26
Total	**82**

Correlation analysis of the C-BARQ for the traits related to aggression

Table 2 shows the correlation between the different factors of the questionnaire. Positive correlations were found between the trait ODA and AAS (r_S=0.44, *P*<0.001), EX (r_S=0.33, *P*<0.01) and ENE (r_S=0.26, *P*<0.05). CHAS was positively correlated to DDA (r_S=0.51, *P*<0.001) and SDA (r_S=0.31, *P*<0.01). SDA was positively correlated with SDF (r_S=0.43, *P*<0.001) and DDA (r_S=0.41, *P*<0.001). DDF was also positively correlated with SDF (r_S=0.41, *P*<0.001).

Table 2. Spearman's correlation coefficient between the different C-BARQ's behavioural traits.

	SDA	ODA	DDF	DDA	TRAIN	CHAS	SDF	NSF	SRB	TS	EX	AAS
SDA												
ODA	0.12											
DDF	0.02	-0.04										
DDA	0.41***	0.18	-0.02									
TRAIN	-0.16	-0.2	0.2	-0.27*								
CHAS	0.31**	-0.22	0.14	0.51***	-0.03							
SDF	0.43***	0.05	0.41***	0.12	0.11	0.28*						
NSF	0.14	-0.03	0.36**	-0.1	0.04	0.18	0.35**					
SRB	-0.13	-0.04	0.11	-0.21	-0.18	-0.07	0.04	0.25*				
TS	0.16	0.07	0.39**	0.03	-0.15	0.24*	0.39**	0.38**	0.28*			
EX	0.21	0.33**	0.07	0.2	-0.19	0.08	-0.07	-0.02	0.28*	0.21		
AAS	0.24*	0.44***	0.17	0.14	-0.2	-0.07	0.26*	0.13	0.23*	0.32*	0.26*	
ENE	-0.06	0.26*	0.08	0.01	-0.03	0.1	-0.02	0.12	0.23*	0.15	0.40***	0.29**

SDA (stranger-directed aggression), ODA (owner-directed aggression), DDF (dog directed fear), DDA (Dog directed aggression), TRAIN (trainability), CHAS (chasing), SDF (stranger-directed fear), NSF (non-social fear), SRB (separation-related behaviour), TS (touch sensitivity), EX (excitability), AAS (attachment or attention seeking behaviour), ENE (energy).

Level of significance: * $p < 0.05$; ** $p < 0.01$;*** $p<0.001$

Discussion

The present study aimed to identify behavioral characteristics related to aggressive behavior through a correlation analysis of the C-BARQ traits so we will discuss correlations related to this trait only.

Owner directed aggression (ODA)

The trait ODA was found to be significantly correlated with AAS (attachment and attention seeking behavior) (r_s=0.44). Considering that attention seeking behavior can be related to a sign of anxiety (Bowen and Heath, 2005), it is possible that this finding is suggesting that some dogs showing owner directed aggression may be suffering underlying anxiety. In fact, many anxious dogs are described as attention seekers (Overall, 1997). These results are in accordance with studies that have described the influence of anxiety on aggression towards family members (Guy et al., 2001b; Reisner, 2003). An association between aggression and anxiety related disorders has been identified in humans as well (Neumann et al, 2010). According to these authors, this relationship is likely to be based on the involvement of overlapping brain pathways regulating emotions and social behaviors.

The fact that a dog that is aggressive towards the owners shows anxiety could be related to different factors. In the first place, this association could be a reflection of a state of motivational conflict or frustration. Studies in laboratory rodents, found that aggressive behavior was facilitated by stress hormones that are secreted in an anticipatory response to the social challenge suggesting a mutual stimulatory interaction between brain mechanisms that control brain areas involved in aggression and the stress response (Kurk et al., 2004). Conflict behavior in aggressive dogs may indicate some degree of stress and uncertainty (Leuscher and Reisner, 2008) which may be caused by owners giving ambivalent signals to the dog thus creating an

environment that is unpredictable and uncontrollable. Inconsistent management (Arhant et al., 2010) as well as punishment-based techniques have been also associated with high number of undesirable behaviors including aggression towards the owners (Blackwell et al., 2008). Although we do not have information related to the training the dogs of this study have received, it is possible that some of them have experienced inappropriate training or punishment, and it has been postulated that aggression could be a consequence of the anxiety developed in the dogs as a consequence of the inappropriate training (Blackwell et al., 2008). Hsu and Sun (2010) also found that dogs subjected to physical punishment scored higher in aggression subscales of the C-BARQ. Such psychological stressors can lead to a dysregulation and overload of the Hypothalamus Pituitary Adrenal axis increasing the risk of aggression (Notari, 2009).

Experiences during early life can also predispose an animal to show aggression (Mertens, 2000) and anxiety (Kibusui et al., 2004). Early weaning mice, for instance, have shown to have higher levels of anxiety than normally weaned animals (Kibusui et al., 2004). Puppies separated from their mothers at 30-45 days were more likely to show behaviours apparently linked to anxiety than puppies that remained with the bitch until 2 months of age (Pierantoni and Verga, 2007). And as we pointed out above anxiety and stress can be linked to aggressive behaviour (Kurk et al., 2004; Notari, 2009). Again, the information about the age of weaning was not included in this study but we cannot discard that have played a role in the results.

The expression of aggressive behavior can also be facilitated by genetic predispositions (Mertens, 2002). Evidence of genetic predisposition can be observed in studies related to behavioral differences between breeds (Scot and Fuller, 1965; Hart and Hart, 1985; Duffy et al., 2008). It has to be taken into account however that not all differences between breeds are the result of genetic variations (Amat et al., 2009). Genuine genetic differences in behavior have been observed in studies that evidence the existence of polymorphisms between different breeds in genes involved in the control

of neurotransmitters like dopamine and glutamate (Niimi et al., 1999; Ogata et al., 2006). In this study we are not able to assess if the differences can be explain by breed differences because the number of individuals representing each breed was too small to make comparisons.

The trait ODA was also positively correlated with the trait EX (excitability) (r_s=0.33) and ENE (energy) (r_s=0.26). Guy et al. (2001a) found that dogs ranking high for excitability in the first two months of ownership were at risk of biting their owners. Excitability is thought to be caused, at least in part, by inconsistent interactions between the owner and the dog, and it could be also associated with a state of anxiety (Arhant et al., 2010).

Stranger directed aggression (SDA)

A significant positive correlation was found between subscale scores for SDA and SDF (stranger- directed fear) (r_s=0.43). This finding is in agreement with the assertion that fear is one of the most common motivational states that can induce aggression towards unfamiliar people (Haug, 2008). Fear related aggression towards unknown people can be the consequence of a lack of contact with people during the socialization period or a traumatic experience with people (Beaver, 2009). Also, similar to other problems related to fear, a genetic predisposition may play an important role (Manteca, 2003). SDA was also positively correlated with DDA (dog-directed aggression) (r_s =0.41). These correlations may indicate that those traits share, at least in part, the same motivational state that could be related to fear. Dog-directed aggression however was independent of dog-directed fear, suggesting that aggression towards other dogs was not motivated by fear. It is true that fear is not the only one motivation for a dog to show aggression towards other dogs, but it has to be taken into account that some dogs may show an offensive posture as a result of learning even when the motivation is fear (Bain, 2009) so the owners may not consider them as fearful to other dogs.

Chasing (CHAS)

CHAS was found to be correlated with two factors related to aggression, DDA (r_s=0.51) and SDA (r_s=0.31). The factor CHAS consists of four items, one indicating the tendency of dogs to show aggression towards small animals and three the tendency to chase them. This factor could be related to different motivations such as predation, lack of stimulation or play, between others. CHAS was also found to be correlated to SDF (r_s=0.28).

As a result of the domestication process, the behavior of the dog has been modified in relation to the wolf (Frank and Frank, 1982). Among these changes, it can be observed that dogs are friendlier towards unknown conspecifics than wolves and they seem to treat them as if they were from the same group. In contrast, as wolves grow up, they become very intolerant towards unknown wolves not belonging to their group and tend to show aggression or fear towards them (Lindsay, 2000). Another aspect of the behavior that has been changed is the predatory behavior, which has been markedly reduced in dogs (Coppinger et al., 1987). Coppinger et al., 1987 hypothesized that some changes caused by domestication vary considerably between breeds of dogs, some breeds having been less modified by the domestication process than others. Regarding our findings, the fact that dogs with a tendency to show predatory behaviors are more likely to show aggression towards unknown dogs could be related to these dogs showing a behavior more similar to that of the wolf than other dogs. Also, the fact that these dogs show aggression toward unknown people, and that this aggression is probably motivated by fear reinforce even more this hypothesis. Due to the small number of dogs of each breed we could not analyze the possible breed effect on behavior. Additional research is needed on this issue.

Conclusions

From the present study, behavioral correlations between aggressive related and other factors were identified. A relationship between owners directed aggression and anxiety is suggested. Stranger directed aggression on the other hand, was found to be related to fear. In addition, there may be differences in predatory behavior and behavior towards unknown dogs and strange people. These findings might help to understand the factors that affect behavior and explain how each individual animal copes with environmental challenges. Also, the identification of the factors associated with aggressive behavior is necessary to improve treatments and to develop prevention strategies. Additional studies are needed with a larger number of dogs.

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CAPITULO II

Individual variations in physiological responses to stress in puppies

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Abstract

The response to stress varies considerably between individuals. The aim of this study was to evaluate the effect of neonatal handling, age, sex and litter on the variability of the stress response in puppies. Seven litters of Beagles were used in this study. Each puppy was randomly assigned to one of three experimental groups: control group (non-handled; NH), handled once a day (HO) and handled three times a day (HT). Neonatal handling was performed from the 3rd until the 21st day of life. At 8 and 12 weeks of age, all puppies were subjected to an Open Field Test (OFT). Saliva cortisol concentration and rectal temperature were measured before and after the OFT. Serum serotonin levels were evaluated on a day different from that when the test was carried out. Serotonin concentration was found to decline with age. No differences between handled and non handled animals were found in any of the variables measured. However, cortisol and rectal temperature were found to be strongly influenced by the litter. Maternal environment is suggested as a possible factor influencing differences between litters.

Introduction

Stress is a biological response elicited when an individual perceives a threat to its homeostasis (Moberg 2000). The stress response includes a series of behavioral and physiological changes. The evaluation of the HPA axis has called much attention when studying the stress response and is probably one of the most frequently used methods to assess how an animal is coping with its environment (Broom & Johnson 1993). The evaluation of cortisol concentrations have been widely used as a measure of the level of stress in dogs. For instance, a significant rise in cortisol concentrations in dogs was found in response to loud noises (Dreschel & Granger 2005), as a consequence of disciplinary behavior by human handlers (Horváth *et al* 2008), as a result of introduction into a novel kennel environment (Rooney *et al* 2007) and in response to short-term unexpected social challenge (Horváth *et al* 2007).

The activation of the HPA axis in the stress response also plays an important role in the activation of the central serotonergic systems. For instance, chronic stress, via the corticotrophin releasing factor (CRF) can induce a change in the serotonergic systems decreasing the density of $5HT_{1A}$ receptors (Leonard 2005). Other studies have demonstrated an association between the HPA axis activity and the serotonin transporter (5-HTT) levels (Reimold *et al* 2011), suggesting a link between stress and anxiety. In dogs, 5 hydroxyindoleacetic acid (5-HIAA), the main serotonin (5 HT) metabolite, measured in the cerebrospinal fluid (CSF) was found to be lower in aggressive dogs in comparison with non aggressive ones (Reisner *et al* 1996). A correlation between blood and CSF serotonergic parameters has been found in humans (Sarrias *et al* 1990) which yielded to the possibility of assessing this neurotransmitter activity with less invasive methods. León *et al* (2010), evaluated the suitability of different types of blood samples for measuring circulating serotonin in canine studies and concluded that sampling serum was the most suitable due to its simplicity.

A high variability in the response to stress has been observed between individuals (Mormède *et al* 2007). Inter individual variability in the HPA axis response can arise from genetic factors (Muráni *et al* 2010) and as a result of early experiences (Nelson 2005), among other factors. Genetics (Fernandez & Gaspar, 2012) and early development (Veenema *et al* 2006) can also explain some differences in the serotonergic system.

Maternal stress during pregnancy, for instance, has been shown to have programming effects on both the neurodevelopment and the function of the offspring's HPA axis (Weinstock 2001), affecting their coping ability. Prenatal stress was also found to alter the distribution of 5-HT terminals in the rat brain, leading to impaired synthesis of hippocampal 5-HT (Peters 1986) and altering the levels of the neurotransmitter and its major metabolite, 5-hydroxyindoleacetic acid (5HIAA) in the hypothalamus and cerebral cortex (Peters 1982).

Experiences during neonatal life can also have profound effects on the response to stress. Neonatal handling is an experimental paradigm known to modify the HPA axis function in such a way that the ability of the organism to respond, cope and adapt to stressful stimuli is increased (Levine 1957). The effects of neonatal handling are mediated by epigenetic modification of gene expression. Neonatal handling increases glucocorticoid receptor expression in the hippocampus and frontal cortex (Meaney *et al* 1985a) and the efficiency of the adrenocortical negative-feedback system (Meaney *et al* 1985b). The serotonergic system can also be affected by neonatal handling. For instance, Papaioannou *et al* (2002), demonstrated that neonatal handling increase the serotonin levels in the brain of rats.

Neonatal handling consists of a brief period of mother – pup separation. Such separation increases the amount of tactile stimulation received by the pups (Priestnall 1973) and this in turn modifies the pups' response to stress later on in. For example, rat pups that receive high amounts of arch-backed nursing (ABN) and licking & grooming (LG) from their mothers exhibit decreased physiological and behavioral responses to stressors, and decreased anxiety (Caldji *et al* 1998; Fish *et al* 2004).

Variability in glucocorticoids and serotonin may also be influenced by gender and age. In laboratory rodents for instance it has been observed that females exhibited higher levels of corticosterone in response to restrain stress than males and that male rats show greater dopamine and serotonin utilization than females (Duchesne *et al* 2009). Differences in serotonin synthesis capacity were also found as a result of age. For instance, in humans, serotonin synthesis capacity was found to be higher in the developing brain than in the adult brain and declined before puberty (Chugani *et al* 1999).

Apart from the evaluation of the HPA axis, the stress response can also be assessed measuring the autonomic response to stress. Stress induced hyperthermia (SIH) refers to the increase of body temperature not linked to organic state of disease and reversed by anxiolitic drugs (Zethof *et al* 1995). SIH have been observed in various species, during exposure to stress-inducing stimuli (Kluger *et al* 1987). In dogs, hyperthermia was found to be part of a fear related autonomic response associated with conditioned stimuli (Ogata *et al* 2006).

The evaluation and identification of the different sources of variability in the physiological response to stress in puppies is of particular interest in laboratory dogs. Such animals can be often subjected to potentially stressful situations and knowing the sources of stress can help to design preventive protocols to ensure the maximum ability to cope, then improving their welfare. Moreover, if the variability is decreased, the animals needed to obtain statistically significant scientific data can be reduced.

Several tests have been proposed to assed the stress response. A widely used test is the open field test (OFT) (Rushen 2000). The OFT consists of a walled and inescapable arena and is aimed to evaluate general emotional response to novelty and isolation (Manteca & Deag 1993). This test was originally developed for rats, but its use has been extended to a great number of species (Prut & Belzung 2003) including cattle (Passillé *et al* 1995), pigs (Donald *et al* 2011), sheep (Pedernera-Romano *et al* 2010), cats (Marchei *et al* 2009) and dogs (Head *et al* 1997).

The aim of this study was to evaluate the effect of neonatal handling, age, sex and litter on the variability of the stress response measuring cortisol concentrations and

rectal temperature in laboratory puppies subjected to an OFT. Moreover, on the basis of the relationship between HPA axis activity and the serotonergic system, we also studied the effect of those factors on serum serotonin concentration. To the best of our knowledge, no studies of these characteristics were performed in dogs before.

Materials and Methods

All procedures were approved by the Ethical Committe of the Departament de Medi Ambient i Habitatge, Serveis Territorials a Barcelona, Àrea del Medi Natural, Generalitat de Catalunya.

Animals and housing

Seven litters of Beagles were used in this study. The average number of puppies per litter was 5.85 ± 0.89 (mean ± SD). The total number of puppies was 41 (22 females and 19 males). All litters were raised in the same breeding kennel, which belonged to an experimental colony. The animals were housed in kennels (3, 75 m^2) placed in the same building. All animals were fed once a day in the morning. The bitches stayed with the puppies until weaning (8 weeks of age) and the puppies remained together until 12 weeks of age. Apart from the treatment, contact with humans was limited to daily cleaning and preventive health procedures.

Each puppy was individually identified by the characteristics of the coat and was randomly assigned to one of three experimental groups. The experimental groups were control group (non-handled; NH) (n=16), handled once a day (HO) (n=14) and handled three times a day (HT) (n=11).

Treatment procedure

Neonatal handling was performed from the 3rd day of birth until the 21st day of life. The handling was carried out by a person who was sitting near the bitch and the other puppies and whose hands were covered with latex gloves. Puppies from groups HO and HT were taken out of the nest individually, placed on an absorbent towel that was in the lap of the handler and stroked with the fingers for 5 minutes. The tactile stimulation involved the entire body of the puppy that was held alternatively in prone and supine position. After handling, the puppies were returned to the nest. Puppies from group HT were handled three times a day with an interval of two hours between sessions. All handling procedures were done six days per week between 9 am to 1 pm.

Open field test

The puppies were tested when they were aged 8 weeks (60.2 ± 1.09 days) and at 12 weeks (86.8 ± 0.83 days). The test was performed in a room where puppies had never been before. All tests were carried out between 10 am and 1 pm.

Each puppy was placed in the same square located in an angle of the arena (1, 94 m x 1, 74 m divided into sixteen 48 cm x 43.5 cm squares) and left alone for 10 minutes. After each trial, the puppy was returned to its kennel and the arena floor was washed with an enzymatic detergent in order to eliminate any odours. The test order of puppies was random.

Physiological measures

Salivary cortisol

Saliva samples were collected from the puppies before and 15 minutes after the end of the OFT (Beerda *et al* 1998). In order to increase saliva flow, the puppies were allowed to smell wet can food for dogs. Saliva was collected by gently rotating a cotton swab inside the dog's cheek for approximately 60 seconds. The soaked swabs were put in Eppendorf tubes and centrifuged (3000 rpm for 15 minutes) to separate the saliva from the cotton. After the separation, the saliva samples were stored in a freezer (-40ºC) until analysed. Salivary cortisol concentrations were analysed using an enzyme immunoassay (ELISA) kit from DRG (Germany).

Rectal temperature

Rectal temperature was measured with a digital thermometer before and after the OFT, but always before taking saliva samples.

Serum serotonin

Blood samples were collected when the puppies aged 8 weeks (60.2 ± 1.81 days) and at 12 weeks (86.8 ± 0.83 days) and were carried out between 12 am and 13 pm. The samples were taken from the jugular vein of each puppy and put into anti-coagulant-free tubes. Samples were centrifuged at 2800 rpm for 15 minutes. Serum was frozen and stored at – 40 ºC until analysed. Serum serotonin was measured using an ELISA kit (DLD Diagnostika GMBH, Hamburg, Germany).

Statistical analysis

Data from cortisol, serotonin, rectal temperature and behavioural measures were analysed using the PROC MIXED procedure of SAS (SAS.9.1.Institute, Inc, Cary, NC) for repeated measures. Significance was fixed at $P<0.05$ in all cases. Normality test of data

and residuals were performed for every variable evaluated. A log transformation was applied to cortisol data. All the variables followed a normal distribution.

The models accounted for the effects of handling, period (8 weeks old vs. 12 weeks old), litter, gender, and type of sample (before or after OFT) in the case of cortisol and rectal temperatures. The model took into account the interactions of period x manipulation and period x litter. The interactions of the type of sample x type of manipulation, type of sample x litter, type of sample x period and type of sample x gender were also considered in the case of cortisol and rectal temperatures. The Tukey Kramer test was used to establish differences between the least means (LSMEANS) of fixed effects.

Results

Concentration of salivary cortisol

Salivary cortisol concentration was significantly higher after (26.50 ± 9.56 ng/ ml) the test than before (19.08 ± 6.57 ng/ ml) (*P <.0001*). Comparisons of the cortisol concentrations showed significant differences between litters (*P*=0.0031). According to the LSMEANS, there was significant differences between one litter (35. 93 ± 10.62 ng/ ml) and two others (17.89 ± 6.32 ng/ ml and 20.38 ± 4.68 ng/ ml) after the OFT. No effect of the handling treatment, gender (*P*=0.29) and period (*P* = 0.83) was found.

Rectal temperature

Rectal temperature was significantly higher after (38.75 ± 0.5ºC) than before (38.2 ± 0.44 ºC) the OFT (*P* <.0001). Significant differences in rectal temperature were found between litters (*P*=0.001). According to the LSMEANS, after the OFT there were significant differences between two litters (38.98 ± 0.42 ºC and 38.18 ± 0.30 ºC). No

differences were found between treatments (*P*= 0.9), gender (*P*= 0.87) or age (*P*= 0.09).

Serum serotonin concentration

Significant differences in serotonin concentration were found between periods (*P* = 0.04), the concentration of serotonin being higher at 8 weeks of age (2206.57 ± 442.89 ng/ ml) than at 12 weeks (1824. 21 ± 419. 41 ng/ ml) of age. No differences were found between treatments (*P*=0.3) or litters (*P* = 0.82). Males and females did not differ in serum serotonin concentration (*P*=0.79).

Discussion

Although we expected to find that non-handled individuals would have an exaggerated stress response in comparison to handled animals, this was not the case, and we also failed to find any difference between the levels of handling. Several studies on the effects of early experiences on emotionality have been carried out in rats and most of them have shown a positive effect of neonatal handling on the stress reactivity (Levine 1957; Ferré *et al* 1995; Beane *et al* 2002). For instance, Meerlo *et al* (1999), found that rats handled during the first 3 weeks of life showed a significantly lower corticosterone (the principal glucocorticoid in the rat) response when tested at 3-4 months age than non-handled animals. Furthermore, rats handled early in life show increased hippocampal type II corticosteroid receptor density and increased sensitivity to the inhibitory effects of circulating glucocorticoids on post-stress HPA activity (Meaney *et al* 1989). One hypothesis that has been suggested to account for the effect of early stimulation is that handling affects the pup indirectly by producing changes in the behavior of the mother (Mason 2000). Thus, it was observed that mothers of handled pups show increased levels of licking and grooming of pups compared with mothers of nonhandled pups (Liu *et al* 1997). Therefore, a possible explanation for our results is

that the separation of the puppies may have not been enough for stimulating the licking behavior of the mother. In rats, ultrasonic vocalizations increased by cooling or tactile stimulation are thought to stimulate maternal behavior (Priestnall 1973; Lee & Williams 1974). It is likely that because of their smaller size in comparison to the puppies, the rat pups loose temperature more easily, vocalizing more and then stimulating the maternal behavior more effectively. By saying that, we are presuming that the smaller the size of the individual handled the better the effect of neonatal handling. Although this hypothesis is intriguing, we would need more studies to confirm it. Piñol *et al* (2005), observed an increase in licking behavior of the bitches after their separation from the puppies. In their study, the fact that the puppies remained without the mother for some minutes may have affected their body temperature in such a way that the maternal behavior was more effectively stimulated.

Another possibility to explain the lack of effect of handling in our study is that all puppies in our study received enough tactile stimulation from their mothers and by the keepers as a result of daily routines –although it was supposed to be minimum- so that any additional stimuli did not have any effect. Another study on neonatal stimulation in dogs did find a positive effect of neonatal handling on some behavioral variables related to emotional stability of 8 week old puppies (Gazzano *et al* 2008). The authors also evaluated heart rate but did not find differences between treatments in this variable. It might be possible that behavioral variables are more sensitive to differences in stress reactivity in puppies than physiological ones. It should also be taken into account that the dogs used in this study were not Beagles and there can be a breed effect on the response to neonatal handling and isolation.

The effect of the litter on salivary cortisol concentration and rectal temperature was highly significant. Because the animals were studied in basal conditions and then exposed to an acute stress it is likely that the difference between litters was related to differences in stress reactivity. Such difference could be explained, at least in part, by differences in maternal behaviour. The effect of maternal care on development has been the subject of various studies in laboratory rodents (Liu *et al* 1997; Caldji *et al* 1998). Individual differences in maternal licking and grooming in rats were found to be

correlated to differences in plasma adrenocorticotropic hormone (ACTH) and corticosterone responses to restrain stress in the offspring when tested as adults (Liu *et al* 1997). Thus the higher the frequency of maternal licking and grooming, the lower the HPA response to stress in adulthood. It is therefore possible that the puppies of bitches that showed higher levels of licking and grooming were less reactive when tested in the OFT.

Other factor that has to be taken into consideration is the role of prenatal stress on the differences between litters. Maternal stress during gestation can affect fetal development by exposure of the fetuses to stress hormones that are transported through the placenta (Weinstock 1997; Bosch *et al* 2007). Data from studies in mammals using different types of gestational stress support the existence of an abnormal regulation of the HPA axis in the adult offspring (Weinstock 2001; Entringer *et al* 2009; Glover *et al* 2010). The fact that the mothers lived under the same environmental conditions does not completely rule out the possibility that some of them may have suffered more stress than others as the degree of stress suffered during gestation is likely to be related to the individual ability to cope with stressful situations.

The effect of the day cannot be discarded as a possible factor influencing the differences between litters, especially the effect of environmental temperature and humidity as they have been identified as potential factors influencing the HPA axis (Marple *et al* 1972).

Finally, genetic variation in stress response has been identified in rodents (Ellenbroek *et al* 2005) and farm animals (Zhang *et al* 1992). Furthermore, studies in rats suggest that the effects of neonatal handling may be different across strains (Durand *et al* 1998). Therefore, genetic factors cannot be ruled out to explain differences between litters.

No gender differences in the stress response were found in this experiment. Studies in adult dogs suggest that females show a higher HPA reactivity to stressors than males (Garnier *et al* 1990; Beerda *et al* 1999). Dreschel & Granger (2005), on the other hand, did not find differences between male and female dogs in the response to stress and

suggested that their results could be due to the fact that the dogs used in the their study were gonadectomized. In laboratory rodents, however, sex differences in the HPA axis were found in neonates (Yoshimura *et al* 2003) which suggests that both the organizational and activating effect of androgens are responsible for the gender differences in stress response. In fact, the presence of testosterone at an early age is thought to be related to the sex-differences in adrenal glands (Yoshimura *et al* 2003). Further studies in dogs in this field are needed to draw final conclusions.

The concentration of serum serotonin was similar in all groups, suggesting that there were no differences between handled and non handled animals. Studies in rodents concerning the effect of neonatal handling on serotonin are contradictory. Papaioannou *et al* (2002) observed an increase in serotonin levels and a decrease in its turnover in three brain regions -hypothalamus, hippocampus and striatum- as a result of neonatal stimulation. This handling induced increase in serotonin seems to be a factor mediating the increase of type II glucocorticoid binding capacity (Mitchel *et al* 1990; Meaney *et al* 2000). However, it is suggested that differences in glucocorticoid receptor expression in adult handled and non-handled animals are not associated with long-term differences in either 5-HT levels or 5-HT_2 receptors (Smythe *et al* 1994) since the role of 5-HT seems to be exclusive of early development. This fact could help to explain why, in our study, differences between litters were found in cortisol concentration but not in serotonin levels.

Similar to studies in humans that found that serotonin synthesis capacity is greater in children than in adults (Chugani *et al* 1999), we found a significant decrease of serum serotonin levels with age. Thus, eight week old puppies had higher concentrations of serotonin than twelve weeks old ones. In humans, serotonin concentration in the cortex is at its highest at two months of age and declines thereafter until 3 years of age (Goldman-Rakic & Brown RM 1982). These changes in serotonin concentration are related to its role as a trophic and neuronal differentiator (Lauder & Krebs 1978).

An increase of both rectal temperature and salivary cortisol concentration was found after the OFT. These results corroborate the negative effect of isolation and novelty in puppies. Puppies were tested twice, at 8 and 12 weeks of age, and although we

expected the stress reaction to be higher the second time as a result of a process of sensitization, this was not seen. Elliot and Scott (Elliot & Scott 1961) studied the reaction to isolation in puppies from 3 to 12 weeks of age and found that it reaches a peak at 6-7 weeks of age and begins to decline thereafter. This factor could have masked the effect of the sensitization in our study.

Conclusion

This work has identified different sources of variation in stress related parameters in puppies. Physiological responses to a stressful event measured by the changes in cortisol concentrations and rectal temperature were found to be strongly influenced by the litter. Maternal environment is suggested as a possible factor influencing these differences. Neonatal handling, age and sex did not show any effect on the stress response. Serotonin concentration was found to be influenced by the age.

Animal welfare implications

The stress response varies considerably between individuals. In laboratory dogs the identification and later reduction of the variability in stress related parameters may help to reduce the number of animals needed to obtain significant scientific data thereby improving overall animal welfare. Furthermore, the identification of factors that reduce or increase the response to stress is important in order to design welfare protocols not only for animals used for scientific purposes but also for companion dogs. In this study, the stress response was found to be strongly influenced by the litter suggesting an important role of maternal environment on it; further research in this area is required.

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CAPITULO III

Individual differences in behavioural responses during the Open Field Test in puppies

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Abstract

The aim of this study was to identify individual differences in the behavioural response to novelty and isolation in puppies and to evaluate if such differences were affected by neonatal handling and by the litter. For this purpose, 41 puppies were randomly assigned to one of three experimental groups: control group (non-handled; NH), handled once a day (HO) and handled three times a day (HT). Puppies of the HO and HT groups were handled between the 3rd and the 21st day of life. At 8 weeks of age, puppies were subjected to an Open Field Test (OFT). Two independent factors were extracted by means of factor analysis. The first factor (Deambulation) had a positive loading for squares crossed (+0.81) and rearing (+0.87) and a negative loading for latency to leave the first square (-0.64), suggesting a high level of activity. The second factor (Reactivity) had positive loadings for time spent in the central square (+0.81) and latency to the first yelp (+0.87). Such differences were found not to be affected by neonatal handling but they were significantly affected by the litter on the first factor. Different hypothesis to explain such differences are discussed.

1. Introduction

The stress response promotes the maintenance of homeostasis and adaptation to physiological and psychosocial challenges of a changing environment. This complex process involves coordinated activation of behavioural, autonomic, and neuroendocrine reactions (Muràni et al., 2010). The way the animal responds depends on a variety of factors including genetics (Ramos et al., 1997) and early life environment (Nelson, 2005), resulting in a high interindividual variability. Postnatal handling, for instance, has been shown to modulate the stress response in such a way that individuals handled in the postnatal period are better able to cope with stressful situations when adults than individuals that have not been handled (Levine, 1957). Thus, it was observed that rats submitted to early handling showed an increase in ambulatory behaviour and a lower frequency of defecation when placed in a novel environment than control animals (Levine et al., 1967). The neonatal handling paradigm was studied for the first time in rats and involves the removing of the pup from the mother for a short period of time provoking a mildly stressful stimulation. It has been observed that when the pup is returned to the dam, the mother spends more time licking it than if they had not been separated (Priestnall, 1973). This increased intensity of maternal care was indeed proposed as a main factor for the physiological changes in handled pups (Lay, 2000). The importance of the role of the mother has also became apparent by studies in rodents that demonstrate the existence of individual differences in maternal behaviour that result in different levels of tactile stimulation provided to the pups. Pups whose mothers presented higher levels of maternal behaviour cope better with stress when adults (Liu et al., 1997). Furthermore, in some line of rats, maternal behaviour was found to be correlated with behavioural response to novelty (Clinton et al., 2007). Such differences can in turn contribute to the emergence of different phenotypes in the offspring. In dogs, the effect of neonatal handling (Fox and Stelzner, 1966; Gazzano et al., 2008) and the influence of the mother on the behaviour of puppies have also been investigated (Scott and Fuller, 1965; Stranbderg et al., 2005; Wilson and Sundgren, 1998). In one

study, the influence of the litter was found to be more important than that of the mother (Stranbderg et al., 2005).

Other factors such as gender differences in the behavioural responses to stress have also been found (Westenbroek et al., 2005). Beerda et al., (1999) found differences between males and females dogs during behavioural challenges, bitches apparently showing higher stress responsiveness than males.

A wide variety of behavioural tests have been proposed to evaluate individual differences in response to stressful situations (Forkman et al., 2007). The Open Field Tests (OFT) was first designed to evaluate emotionality in rats measuring defecation (Hall, 1934) and level of ambulatory activity as an index of fear (Hall, 1936). Since then, the OFT has been widely used as a standard technique in many domestic species including cattle (De Pasillé et al., 1995), pigs (Donald et al., 2011), sheep (Pedernera-Romano et al., 2010), cats (Marchei et al., 2009) and dogs (Head et al., 1997). The test consists of the measurement of behaviours elicited by placing the animal in a novel open space from which escape is prevented by a surrounding wall (Walsh and Cummins, 1976). The stressful reaction is thought to be a result of different factors such as the removal of the individual from a familiar home environment, the stimulation involved in transferring the animal to the open field, the exposure to the novel environment and social isolation (Walsh and Cummins, 1976). The response of the animal during the test can be influenced by its coping style and many independent variables such as sex, age and early experiences (De Pasillé et al., 1995). In dogs for instance, activity during the OFT was found to be affected by age (Head et al., 1997; Siwak et al., 2002), pharmacological intervention (Siwak et al., 2000), breed (Head et al., 1997) and cognitive impairment (Rosado et al., 2012), among other factors. Other studies in dogs have shown that fear can lead to modification of locomotory behaviour during the OFT (Landsberg et al., 2009) while in other this variable was not affected by fear (Araujo et al., 2010). Altogether, this findings reinforce the idea that the behaviours evaluated in the OFT can be subjected to a high variability and that

conclusions may not be drawn from a single behaviour, especially when evaluated fear response. For these reasons, a multiple dimensional approach such as factor analysis and multiple testing are proposed in order to facilitate the interpretation (Ramos and Momèrde, 1998).

The aim of this study was to identify individual differences in the behavioural response to novelty and isolation in puppies and to evaluate if such differences were affected by early experiences, in particular by neonatal handling and the litter. To the best of our knowledge, neonatal handling –from birth to 21 days of life- in dogs is not a routine practice in most dog breeding colonies. The evaluation of its potential benefits would provide useful information to design welfare protocols to facilitate the adaptation of the animals to potentially stressful situations. The effect of gender on the response to novelty and isolation was also investigated. Factor analysis was used for the reduction of data and to identify individual differences in the way the animals respond to stress.

2. Materials and Methods

All procedures were approved by the Ethical Committe of the Departament de Medi Ambient i Habitatge, Serveis Territorials a Barcelona, Àrea del Medi Natural, Generalitat de Catalunya.

2.1. Animals and housing

Five litters of Beagles were used in this study. The average number of puppies per litter was 5.85 ± 0.34 (mean ± SE). The total number of puppies was 41 (22 females and 19 males). All litters were raised in the same breeding kennel, which belonged to an experimental colony. The animals were housed in kennels (3. 75 m^2) placed in the same building. The bitches stayed with their puppies until weaning (8 weeks of age)

and the puppies remained together until 12 weeks of age. Apart from the treatment, contact with humans during the neonatal period – from birth until the 21st day of life- was limited to daily cleaning and preventive health procedures.

Each puppy was individually identified by its coat characteristics and was randomly assigned to one of three experimental groups: control group (non-handled; NH) (n=16), handled once a day (HO) (n=14) and handled three times a day (HT) (n=11).

2.2. Treatment procedure

Neonatal handling was performed from the 3rd until the 21st day of life. Handling was carried out by a person who was sitting near the bitch and the other puppies and whose hands were covered with latex gloves. Puppies from HO and HT groups were taken out of the nest individually, placed on an absorbent towel that was in the lap of the handler and stroked with the fingers for 5 minutes. The tactile stimulation involved the entire body of the puppy, which was held alternatively in prone and supine position. After handling, the puppies were returned to the nest. Puppies from HT group were handled three times a day with an interval of two hours between sessions. All handling procedures were done six days per week between 9:00 am to 1:00 pm.

2.3. Behavioural tests

The puppies were tested by means of an OFT when they were 8 weeks old (60.2 ± 0.48 days old). The test was performed in a room where puppies had never been before. All tests were carried out between 10:00 am and 1:00 pm.

The arena measured 1. 94 x 1. 74 m and was divided into sixteen 48 x 43.5 cm squares. Each puppy was placed in the same square located in an angle of the arena and left alone for 10 minutes. After each trial, the puppy was returned to its kennel and the arena floor was washed with an enzymatic detergent in order to eliminate olfactory

cues from the previous puppy. The test order of puppies was random. The description of the behaviours is summarized in Table1.

Table 1

Ethogram of the behaviors recorded in the open field.

Behavior	Definition
Squares crossed	Number of squares (n=16) entered by the puppy (with its front paws) during 10 min test period.
Rearing	Puppy is scratching or climbing the wall, or is rearing with its front paws on the wall
Time spent in the central square	Time spent in the centre of the arena (n=4 squares)
Latency to leave the first square	Time to leave the square where the puppy was placed at the beginning of the test
Latency to the first yelp	Time to emit the first vocalization

2.4. Statistical analysis

A factor analysis using Proc Factor procedure of SAS (SAS.9.1.Institute, Inc, Cary, NC) was performed considering total duration of walking and time spent in the central square, frequency of escape attempts and latencies to leave the first square and latency to emit the first vocalization. The contribution of a given variable to a given factor was defined by its loading value and the minimum absolute value of loading factors was set at 0.30. After extraction of the initial factor solution, an oblique rotation (PROMAX) was performed in order to simplify the view of the structure of the data being analyzed. Factor scores were then calculated and subsequent analysis was performed on these scores using the GLM procedure of SAS. The model accounted for the effects of handling, gender and litter. A Tukey Kramer test was used to establish

differences between the least means (LSMEANS) of fixed effects. Significance was fixed at $P<0.05$ in all cases.

3. Results

When puppies were tested at 8 weeks of age, two independent factors were revealed by means of a factor analysis, which together accounted for the 46.11 % of the total variance of the five behavioural variables (32.27% for Factor 1 and 13.74% for Factor 2). Even though the eigenvalue of the second factor was less than 1 (0.68), its inclusion increased the total variance. The first factor (Deambulation) had a positive loading for squares crossed (+0.81) and rearing (+0.87) and a negative loading for latency to leave the first square (-0.64). On the other hand, the second factor (Reactivity) had positive loadings for time spent in the central square (+0.81) and latency to the first yelp (+0.87). Factor loadings for each factor are shown in table 2.

Table 2

Factor loading for the 2 extracted factors.

Measures	Factor 1	Factor 2
Squares crossed	0.81	0.13
Escape attempts	0.87	-0.25
Time spent in the central square	0.12	0.81
Latency to leave the first square	-0.64	-0.28
Latency to the first yelp	-0.09	0.87

Scores for the two factors were unaffected by gender (*P*=0.20) and treatment (*P*=0.60). Significant differences between litters were found in Factor 1 (*P*=0.01). Litter one was found to be different from litter three (Fig.1). Scores from Factor 2 were unaffected by the litter.

4. Discussion

In this study, an OFT was used to evaluate the effect of neonatal handling on the behavioural responses of puppies. Puppies were tested at 8 weeks of age and two independent factors were revealed by means of a factor analysis. The first factor had a positive loading for squares crossed and rearing and a negative loading for latency to leave the first square, suggesting a high level of locomotor activity. The second factor had positive loadings for time spent in the central square and latency to the first yelp. In descriptive terms, two behavioural patterns could be distinguished. Some animals came out from the first square very fast and walk back and forward and rear or jump over the walls. This behaviour directed to the walls is probably linked to thigmotactic behaviour as the animals may be looking for a way to escape (Marchei et al., 2009). The thigmotaxis is considered an index of anxiety (Simon et al., 1994) but it also seems to be linked to the active or passive response pattern adopted toward challenging situations (Marchei et al., 2009). Some individuals, on the other hand, started walking from the first square very slowly and spent a lot of time in the central square. As can be seen in the plot, puppies of factor 2 can de differentiated on basis of their reactivity. Harri et al. (1995) studied the behavioural reactions of foxes during an OFT and extracted two independent factors that are partially in accordance to our results. The first factor was related to general activity and included mainly locomotion while the second one loaded for ambulation in the central part of the arena especially at the beginning if the test. In other study aiming to evaluate the effect of different rearing conditions on the exploratory behaviour of puppies, two factors were identified at 8.5 weeks of age (Wright, 1983). The variables that contribute to first factor were related to locomotor activity which is also similar to our results. The second factor was called

"stimulus reactivity" and its variables were related to exploration of objects. Comparisons with this second factor are difficult since we did not utilize objects in the arena.

One aim of our study was to evaluate if the individual differences in the behavioural response to stress detected during the OFT were affected by the handling of the puppies during the neonatal period. No effects were found between handled and non handled animals. In their early study, Fox and Stelzner (1966) found differences in behaviour between handled and non handled puppies tested at 5 weeks of age. When comparing these results with the present study, it has to be taken into account that considerable differences exist in their experimental design. The handling procedure in Fox and Stelzner's experiment, for example, consisted on one hour stimulation per day (versus 5 to 15 minutes in our study) and the stimulation comprised many situations, not only stroking the puppies. In addition, they stimulated their puppies from birth until 5 weeks of age while we only did so until the end of the transition period (3 weeks of age). However, in a more recent study in which the puppies were stroked 5 minutes a day until 3 weeks of age, the authors did find differences between handled and non handled puppies (Gazzano et al., 2008). The discrepancy between this study and ours may be due to differences in the type of open field, the parameters measured and the way the variables were analysed. Gazzano et al. (2008), for example, analysed the behavioural variables separately and did not make a reduction of the variables measured during the arena test by means of factor analysis as we did. It should be taken into account also that the breeds of dogs used in both studies were different. In some strains of laboratory rodents, for instance, the handling during the neonatal period was found not to be effective to produce differences in the behavioral response to stress (Durand et al., 1998). Head et al. (1997) also found breed differences in dogs regarding some parameters measured during the OFT.

Individual differences in the response to novelty and isolation were found between litters. Thus, litter one was found to be significantly different from litter three. When

observing the plot, it can be seen that litter three is much more disperse than litter one that is more homogeneous. The greater variability of litter three may be related to a lower capacity to cope with a stressful situation than litter one. Such differences may be related to genetic factors. Although all dogs belonged to the same breed, they could well belong to different strains and this may have influenced the results. Indeed, as pointed out above, strain differences in rodents may influence the effect of neonatal manipulation (Durand et al., 1998) and it is possible that the same phenomenon happens in dogs. Additionally, genetic factors can also influence coping strategy (Koolhass 1999; Marchei et al., 2011). Differences between litters may have been influenced by the behaviour of the mother as well. As was mentioned in section one (Lui et al., 1997), studies in rodents have shown that individual differences in maternal behaviour during the first weeks of life can contribute to individual differences in the ability to cope with stress in the offspring (Caldji et al., 1998; Menard et al., 2004). For example, when rats are placed in a novel environment, High Responder animals show an active exploratory behaviour while Low Responders show a blunted locomotory response, and such differences have been observed to correlate with differences in maternal behaviour, which may thus contribute to the emergence of behavioural phenotypes in the offspring (Clinton et al., 2007). It is therefore possible that the differences between litters observed in our study are related to differences in maternal behaviour and response to novelty. In dogs, there is some evidence indicating genetic and direct behavioural influences of the mother on the behaviour of their offspring (Scott and Fuller, 1965; Wilson, 1984; Wilson and Sundgren, 1998). For instance, differences in behaviour of puppies subjected to a test were found to be affected by heredity factors based on dam variance (Wilson and Sundgren, 1998). Wilson, 1984 observed differences in the behaviour of the bitches in weaning behaviour and suggested that such differences may affect submissive behaviour and trainability. In one study, the litter environment was found to be more important than the role of the mother (Strandberg et al., 2005). Litter environment may include many factors such as temperature and humidity. The level of maturation cannot be discarded as a possible factor influencing the differences between litters and have to be taken into account. Differences in maturation between litters is expected to be

higher between litters than within (Wilson and Sundgren, 1998) and may affect the variation between them them.

No sex related differences were found in either of the two factors at any age. These findings are in accordance with those of Wright (1983) who did not find differences between males and females in puppies tested at 5 and 8.5 weeks of age. In another study, Beerda et al. (1999) found differences between males and females when confronted to another challenges but not during the OFT, suggesting that there may be gender differences in the perception of different challenges. It should be noticed also that the dogs tested in the study by Beerda et al. (1999) were adults while the animals used in ours were puppies suggesting a possible age effect.

5. Conclusions

Through the present preliminary study we have identified individual differences in the behavioural response to a stressful response in puppies during the OFT. Such differences were found not to be affected by neonatal handling. However, significant differences between litters were found and it is hypothesized that genetics, the behaviour of the mother and other factors related to the litter environment may have played a role in such differences. Additional studies are needed to clarify the role of the mothers on the behavioural phenotypes of the puppies.

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CAPITULO IV

Canine aggression towards family members: clinical presentation and causal factors

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Abstract

The aim of the present retrospective study was to investigate the main features of cases of canine aggression towards family members in a referral practice and to determine the causal factors related to this problem. Forty three cases of canine aggression towards family members seen at the Animal Behaviour Clinic (Barcelona School of Veterinary Medicine) were analysed and compared with 50 control cases (dogs without canine aggression towards family members). Dogs adopted after 12 weeks of age presented a lower risk of being aggressive towards household members (P=0.01). Being fed from the table was also considered as a potential causal factor (OR=8; IC=2.4-27.9) for showing aggression towards the owners. Dogs presenting an underlying painful condition had also a higher risk of being aggressive towards family members (OR=14; IC=1.3-165). Dogs were aggressive in competitive contexts (74.4%), in response to frustration (55.8%) and as a defensive reaction (48.83%) and more than half of the dogs (55.81%) were aggressive in more than one context. According to the owner's description, the postures adopted by the dogs during the aggressive events were defensive (27.5%), offensive (15%) and ambivalent (53.48%). Two individuals (4.6%) showed impulsivity (i.e. lack of warning signals prior to the attack) in all aggressive events, 11 dogs (25.5%) were impulsive sometimes but not always and the rest of the dogs (69.7%) always showed warning signals. The possible practical implications of these findings are discussed.

Introduction

Canine aggression is the most frequent complaint in veterinary behaviour referral practice (Overall, 1997; Fatjó et al., 2006; Fatjó et al., 2007; Borchelt, 1983) and aggression directed towards family members is the most common type (Fatjó et al., 2007). Canine aggression towards family members can adversely affect the human animal bond. Statistical studies of dog bites to humans indicate that in most cases people are victims of their own dog or of a dog they know (Guy et al 2001c; Rosado et al., 2009; Wright, 1990). Not only the owners can be affected by this problem but also the welfare of the dog itself can be severely compromised.

Many cases of aggression, for example, may result from a negative emotional state and are often related to a stress response (Kurk et al., 2004). Furthermore, dogs presenting aggression are at a higher risk of being abandoned (Salman et al., 1998; Salman et al., 2000) or even euthanized only for this reason (Overall, 1997).

Aggression, as well as other behaviour problems, can be influenced by genetics and environmental factors. Evidence of genetic effects on aggressive behaviour has been found in some studies that have detected a breed effect (e.g. Scott and Fuller, 1965; Hart and Hart, 1985; Amat et al., 2009). Environmental and management factors have also found to influence aggressive behaviour, although some contradictory findings are observed in different studies. For instance, feeding the dogs directly from the table was found to be a predictor of a dog biting a person in one study (O'Sullivan et al., 2008), but on the other hand, Voith et al., (1992) failed to find a relationship between this activity and other activities they defined as anthropomorphic such as letting the dog sleep in the owner`s bed and the prevalence of behaviour problems in general. The influence of training was also evaluated. Obedience training was found to be related to a reduced incidence of competitive aggression (Jagoe and Serpell, 1996). However, the use of punishment was associated to aggression (Arhant et al., 2010; Tami et al., 2008) and other behavioural problems (Hiby et al., 2004).

The effect of sexual hormones was also considered a factor related to aggressive behaviour. Thus, the influence of testosterone in the expression of offensive type of aggression has been recognised in many animal species including the dog (Manteca, 2003; Nelson, 2005). In fact, many studies have found that males are overrepresented in the population of aggressive dogs (Amat et al., 2009; Borchelt, 1983; Fatjó et al., 2007; Reisner et al., 2005).

Finding out the motivation of aggressive behaviour is important to understand the problem, to prevent it and to implement a successful and safe treatment. For many years, canine aggression towards owners has been linked to dominance, where the dog reacted aggressively when it perceives a challenge from a subordinate (Cameron, 1997). Currently, most authors agree that there is not enough evidence to support the hypothesis that dogs establish hierarchical relationships with its owners (Bradshaw et al., 2008; De Keuster and Hildegard, 2009). Furthermore, the influence of anxiety and conflicting motivations in cases that were formerly attributed to dominance aggression has been recognized in the last years (Reisner, 2003; Leuscher and Reisner, 2008).

The aim of this retrospective study was to further investigate the main features of cases of canine aggression towards family members in a referral practice and to determine the causal factors related to this problem. The characteristics of the attacks were also investigated in order to assess the possible motivations that lead the dogs to show aggression. Much information exists in the literature regarding causal factors related to aggression, yet many things remain unclear. The identification and understanding of the causal factors and motivations are essential for implementing preventive measures and treatment protocols.

Materials and Methods

We reviewed the characteristics of 93 cases of dogs presented at the behavioral service of the Veterinary Hospital of the Autonomous University of Barcelona, Spain. These cases were categorized into two groups “aggressive towards family members” (AGR) (n=43) and “non aggressive towards family members” (NonAGR) (n=50). The selection of the cases was random. The clinical history had been obtained through a standard questionnaire to be filled in by the owner and providing general information and a detailed description of the dog’s behaviour. The variables selected from the questionnaire are summarized in table 1. A physical and neurological examination was performed on all dogs.

Table 1 Variables selected from the questionnaire

Variables	Categories
Characteristics of the dog	
Gender	Male
	Female
Neutering status	Neutered
	Entire
Weight	Less than 10 kg
	More than 10 kg
Age of acquisition	Birth to less than 4 weeks of age
	Four weeks to less than 12 weeks of age
	Twelve weeks onward
Origin	Breeder
	Particular
	Shelter
	Pet shop
	Street
	Unknown
Presence of a painful condition	Yes/ No
Environment and management	
Access to a garden	Yes/ No
Frequency of walks	Null
	One to 3 times per day
	More than 3 times per day
Total amount of time in walks	Less than 1 hour per day
	One to 2 hours per day
	More than 2 hours per day

Feeding regime	*Ad libitum* Restricted
Does the dog receive treats when the owners are eating?	Yes/ No
The dog is allowed to get on the sofa	Yes/ No
The dog is allowed to sleep in owner´s bed	Yes/ No
Training methods	Positive reinforcement only Positive reinforcement and consistent punishment Positive reinforcement and inconsistent punishment
<u>Other behavioural problems</u>	
Aggression towards unfamiliar people	Yes/ No
Aggression towards dogs	Yes/ No
Non social fears or phobias	Yes/ No

To evaluate the characteristics of the aggressive episodes in dogs of the AGR group, four contexts were considered: Competitive (aggression occurred when a person challenged the dog over a resource such as food or toys), Frustration (aggression occurred when the dog was denied to perform a certain behavior or failed to obtain something it wanted), Defensive (aggression occurred when the dog was pushed to accept or do something or in response to punishment). We also analyzed the postures adopted by the dogs during the aggressive episodes: Offensive (raised tail, pricked up ears, eyes fixed to the objective, and straight forelegs during the attacks), Defensive (tail between legs, fallen ears, averted sight and folded forelegs), Ambivalent (mixture of offensive and defensive elements) and Predatory (stare and stalk at the person or silently pursuing it). The reduction or complete lack of warning signals previous to an attack, considered as impulsiveness, was also taken into account.

A logistic regression model was applied to detect possible causal factors. Variables were taken forward for multivariable analysis when significant at $P < 0.2$. Stepwise backward selection was performed to identify the variables that had a significant association ($P < 0.05$) with the outcome measure. The possible relationship between the variables was analysed by means of a chi-square test when the variability of the data did not allow a correct modeling. A P value of 0.05 was considered significant for all analyses. The data was analyzed using the statistical package SAS (SAS.9.1.Institute Inc., Cary, NC, USA).

Results

The average age at the time of consultation was 3.29 ± 0.39 (mean ± SE) years for AGR dogs and 3.46 ± 0.49 years for NonAGR dogs. Among dogs of the AGR group (n=43), 12 (27.9%) were females (66.6% of which were intact) and 31 (72.1%) were males (64.5%

of which were intact). In the NonAGR group (n=50), 23 (46%) were females (65.12% of which were intact) and 27 (54%) were males (25.9% of which were intact). No significant differences were found neither between males and females (P=0.1) nor between neutered and intact animals (P=0.7) in the AGR and NonAGR groups. Twenty eight (68.3%) of the AGR dogs and 34 (72.3%) of the NonAGR dogs weighted more than 10 kg. No significant differences were found between the two groups. The prevalence of dog aggression toward household members is summarized according to the set of selected causal factors in Table 2.

Table 2 Characteristics of the AGR and control dogs

Variables	**AGR group**	**NonAGR group**
Characteristics of the dog	N (%)	N (%)
Gender		
Male	31 (72.1%)	27 (54%)
Female	12 (27.9%)	23 (46%)
Neutering status		
Neutered	15 (34.9%)	15 (30%)
Entire	28 (65.1%)	35 (70%)
Weight		
Less than 10 kg	13 (31.7%)	13 (27.7%)
More than 10 kg	28 (68.3%)	34 (72.3%)
Age of acquisition		
Birth to less than 4 weeks of age	10 (24.39%)	2 (4.34%)
Four weeks to less than 12 weeks of age	23 (56%)	18 (39.13%)
Twelve weeks onward	8 (19.51%)	26 (56.52%)
Origin		
Breeder	12 (27.9%)	14 (28%)
Particular	12 (27.9%)	14 (28%)
Shelter	2 (4.7%)	8 (16%)
Pet shop	7 (16.3%)	2 (4%)
Street	7 (16.3%)	5 (10%)
Unknown	3 (7%)	7 (14%)
Presence of a painful condition		
Yes		1 (2%)

No	6 (16.2%) 31 (83.8%)	49 (98%)
Environment and management		
Access to a garden		
Yes	16 (37.2%)	14 (28%)
No	27 (62.8%)	36 (72%)
Frequency of walks		
Null	5 (11.6%)	4 (8%)
One to 3 times a day	32 (74.4%)	33 (66%)
More than 3 times per day	5 (11.6%)	8 (16%)
Total amount of time in walks		
Less than 1 hour a day	14 (32.6%)	10 (20%)
One to two hours a day	21 (48.8%)	27 (54%)
More than 2 hours a day	6 (14%)	9 (18%)
Feeding regime		
Ad libitum	18 (43.9%)	13 (26.7%)
Restricted	23 (56.1%)	33(71.7%)
Does the dog receive treats when the owners are eating?	24 (60%)	18 (43.9%)
Yes	16 (40%)	13 (28.3%)
No		
The dog is allowed to get on the sofa		
Yes	15 (41.7%)	21 (58.3%)
No	21 (46.7%)	24 (53.3%)
The dog is allowed to sleep in owner's bed		
Yes	14 (35%)	26 (65%)
No	10 (23.3%)	33 (76.7%)
Training methods		
Positive reinforcement only	2 (5.4%)	10 (20.8%)
Positive reinforcement and consistent punishment	16 (43.2%)	23 (47.9)
Positive reinforcement and inconsistent punishment	19 (51.4%)	15 (31.2%)
Other behavioural problems		
Aggression towards unfamiliar people		
Yes	21 (48.8%)	12 (24%)
No	22 (51.2%)	38 (76%)
Aggression towards other dogs		
Yes	22 (52.4%)	20 (47.6%)
No	20 (40.8%)	29 (59.2%)
Non social fears or phobias		
Yes	16 (39%)	19 (38%)
No	25 (61%)	31 (62%)

Dogs adopted after 12 weeks of age presented a lower risk of being aggressive towards household members (P=0.01). The mean age of adoption of each category was 2.9 ± 0.40 weeks of age for dogs acquired with less than 4 weeks of age, 7.88 ±0.31 weeks of age for dogs adopted in the period between 4 to 12 weeks of age and 22.54 ± 2.47 weeks of age for dogs acquired after 12 weeks of age. When comparing the independent variables, a relationship between the age of adoption and the training methods used by the owners was found (χ^2 =10.8, P=0.02). Thus, dogs adopted at 12 weeks of age or more were more likely to be trained using positive reinforcement only.

Dogs presenting an underlying painful condition had also a higher risk of being aggressive towards family members (OR=16; IC=1.53-175.62), P=0.021. Also, aggressive dogs suffering from a painful condition were more likely to be impulsive (P=0.044) than aggressive dogs not presenting a painful condition. Being fed from the table was also considered as a potential causal factor (OR=6.52; IC=2.08-20.4) P=0.001 for showing aggression towards the owners.

No significant differences were found between the other variables studied.

Dogs were aggressive in competitive contexts (74.4%), in response to frustration (55.8%) and as a defensive reaction (48.8%) and more than half of the dogs (55.8%) were aggressive in more than one context. According to the owner's description, the postures adopted by the dogs during the aggressive events were defensive (27.5%), offensive (15%) and ambivalent (53.5%). Two individuals (4.6%) showed impulsivity (e.g. lack of warning signals prior to the attack) in all aggressive events, 11 dogs (25.5%) were impulsive sometimes but not always and the rest of the dogs (69.7%) always showed warning signals.

Discussion

In this retrospective study we aimed to evaluate the main features of dogs showing aggression towards family members seen in a referral practice and to determine some causal factors related to this problem.

We found that dogs adopted after 12 weeks of age presented a lower risk of being aggressive towards their owners than dogs adopted before that age. This finding may be related to various aspects. In the first place, it has to be considered that in many species, including the dog, it has been observed that early weaning can have detrimental effects on the behaviour of the offspring. Early weaned mice, for instance, were found to present higher levels of anxiety than normally weaned animals (Kibusui et al., 2004). In other study with dogs, it was observed that puppies separated from their mothers at 30-45 days were more likely to show behaviours apparently linked to fear and anxiety than puppies that remained with the bitch until 2 months of age (Pierantoni and Verga, 2007). Weaning represents a process where the mother starts to refuse the puppies' attempts to nurse. In dogs, weaning starts at about 4 to 5 weeks of age (Wilson, 1984) and it comes to an end somewhere around 10 to 12 weeks of age (Overall, 1997). The interaction between the mother and offspring during this process is likely to have permanent effects on the behaviour of the puppies (Wilson, 1984). In cats, it was also hypothesized that during the interaction with the mother the offspring learns to tolerate frustration better (Rochtliz, 2005). Thus, it would be expected that dogs weaned at an early age are predisposed to suffer from anxiety related behaviour problems and to be less tolerant to frustration. Anxiety and frustration can be linked to aggressive behaviour towards owners (Bowen and Heath, 2005; Leuscher and Reisner, 2008). It would appear then that puppies should remain with their mothers at least until the end of the weaning period.

This may explain why in our study puppies adopted after 12 weeks of age had a lower risk to

present aggression towards their owners than puppies adopted before 4 weeks of age, however it does not completely explain why they have a lower risk than puppies adopted between 4 and < than 12 weeks of age. The mean age at the time of adoption in that group of puppies was at 7.88 ± 0.31 weeks of age, which is an appropriate time for adoption. This suggests that the possible benefits of adopting from 12 weeks of age onwards in comparison to adopting between 4 and 12 weeks of age may be related to different factors but the interaction with the mother.

The fact that dogs adopted at 12 weeks of age were less likely to be aggressive could be related to owners attitudes towards the dogs as we also observed that dogs adopted from 12 weeks onwards were less likely to be trained using punishment. The use of punishment was found to be associated with aggressive behaviour (Arhant et al., 2010; Blackwell et al., 2008; Tami et al., 2008) while the use of positive reinforcement alone was associated with the lowest mean score for aggression (Blackwell et al., 2008). Then it is possible that owners who adopt a dog that is older tend to be better informed about dog behaviour and training than people adopting a younger one. Such kind of owners may also interpret more accurately dog signals and respond more appropriately to them.

The finding that dogs adopted after 12 weeks old were less prone to show aggression towards their owners contradicts the commonly held belief that owners should adopt a puppy before the end of the socialization period (12 weeks of age) in order to ensure that the puppy will receive enough stimulation and specially to prevent problems related to fear. Serpell and Jagoe, 1995, for instance reported a linear increment of the prevalence of fear to other dogs and fear of traffic with the age of acquisition. Then it is possible that the adoption after 12 weeks of age may increase the risk of developing some kinds of behavioural problems, but not necessarily aggression

towards the owners. Takeuchi et al., 2001, for instance, found that the mean age of dogs with aggression towards owners (0.2 years) when they were first obtained was significantly lower than that of dogs with separation anxiety (0.6 years) and that of dogs that were aggressive towards unknown people (0.3 years). It has to be considered also that the control group of our study included dogs suffering from some behavioural problem different from aggression toward owners. Also, it has to be taken into account that the risk of developing a behavioural problem when adopted after the socialization period is expected to be related to the conditions in which the puppy is raised and if the puppy receives enough stimulation before weaning, late weaning may not be a risk factor.

It was observed that dogs receiving treats from the table had a significant higher risk of presenting aggression towards family members. This result is in accordance with that of Sullivan et al., 2008 who found that dogs with a history of biting a person were more likely to be fed from the table during mealtimes. The relationship between giving treats from the table and aggression towards the owners can be viewed in two different ways. First, this relationship could be an evidence of an inconsistent management of the owner, especially if the treats are given in a random way. Such non-contingent kind of reinforcement deprives the animal of a sense of control over its environment because the dog does not know which behaviours will elicit a reward (Mills, 2009). Lack of consistency on the interactions and unpredictability have been found to be associated with aggressive behaviour (Arhant et al., 2010). In other studies, it was observed that trained dogs were less likely to be fed between their regular meals (Voith et al., 1992) and obedience training was found to be associated with a lower prevalence of aggression towards the owners (Jagoe and Serpell, 1996). It is possible then that the fact that dogs receiving food from the table were more prone to show aggression can be related to a lack of training in such dogs. Obedience training without using aversive techniques such as punishment can help to create a controllable and predictable environment (Leuscher and Reisner, 2008). Voith et al., 1992 however, failed to found an association between feeding from the table and the incidence of behavioural problems. This contradictory finding may be explained at

least in part by the fact that the population of that study included dogs with many behavioural problems apart from aggression towards owners.

In our study, however, we did not find differences in the food regime between aggressive and non aggressive dogs. This finding is in agreement with Podberscek and Serpell (1997) and Guy et al. (2001a) that reported that the feeding regime was not significantly associated with aggressive behavior.

Jagoe and Serpell (1996) observed that dogs allowed to sleep close to the owner were predisposed to refrain from being aggressive n when attention was given to others dogs. We did not find significant differences between dogs allowed to sleep on the owner's bed or allowed to get on the sofa and dogs that were not allowed to do so. It is likely that the owner of an aggressive dog may not feel comfortable sleeping with the aggressive dog or allowing it onto the sofa It is not possible with these results to determine if our finding is a consequence or if there is no direct association between these practices and aggression towards owners.

According to our results, dogs having a painful condition were at higher risk of presenting aggression towards family members. Pain has been associated to aggressive behaviour in dogs (Beaver, 2009; Camps et al., 2012) and is likely to represent a defensive reaction to avoid physical contact that may cause further injury (Rutherford, 2002). Anticipation of pain as a result of a previous experience may also provoke the same reaction (Mertens, 2002). Moreover, the stress response elicited by a chronic painful condition can lead to changes in the central nervous system, such a reduction of serotonin activity (Mellor et al., 2000). In dogs, 5 hydroxyindoleacetic acid (5-HIAA), the main serotonin (5 HT) metabolite, measured in the cerebrospinal fluid (CSF) was found to be lower in aggressive dogs in comparison with non aggressive ones (Reisner et al., 1996). Individuals suffering from pain are also more likely to reduce the level of exercise which can also lead to a reduction in brain serotonin levels (Chaouloff, 1997).

In the study of Camps et al., 2012, osteoarthritis, especially due to hip dysplasia, was found to be the most common cause of pain in a group of dogs showing pain aggression. Consequently, hip dysplasia was considered as a potential risk factor for developing aggression in dogs.

Aggressive dogs that suffered from a painful condition were found to be more likely to be impulsive. In aggressive contexts, the term impulsiveness has been described as a lack of warning signals prior an attack (Peremans et al., 2003). In our study, we observed that 4.6% of the dogs showed always unpredictable attacks –without giving warning signals- while 25.5% gave warning signals only sometimes. These percentages are in accordance with the study of Amat et al., (2009) that found that 6 and 37% of dogs were always or sometimes impulsive. The impulsive aggressive behaviour seems to have a different biological basis as compared with appropriate aggressive responses. Thus, impulsive behavior has been linked to decreased levels of serotonin (Reisner et al., 1996; Wright et al., 2012), to a learning process (Pageat, 1998) and to morphological traits particular to certain breeds of dogs that may complicate the identification of warning signals (Goodwin et al., 1997). The fact that dogs with pain showed less warning signals before the aggressive attack could be related to an anticipation of an aversive experience due to a learning process. Any dog can learn to anticipate an unpleasant situation but in the case of dogs that are in pain, this learning process may be more quick and intense as pain considerably reduces the threshold for aggressive behavior and increases irritability and self defensiveness (Bowen and Heath, 2005).

One of the aims of this study was to investigate the motivations of dogs showing aggression. In order to do this, we evaluated the contexts in which aggression occured and the postures adopted by the dogs during the aggressive events. Regarding the context, we observed that most of the dogs reacted in a context of competition for a resource but they presented aggression also in response to frustration and as a defensive reaction. Even though the context may help us to understand the motivation

of the dog to react in an aggressive way, the interpretation is no easy, especially because it is likely that the dog may be experiencing different motivations at the same time. For example, a dog that is aggressive in competitive contexts may be defending a resource that it perceived as being threatened (De Keuster and Hildegard 2009). However, at the same time, it may experience frustration if the resource is taken away and also may be showing defensive behaviour if previous punishment has been applied in the same context.

For many years, most cases of dog's aggression towards family members have been associated to an underlying hierarchical conflict between the dog and the family members (Cameron, 1997; Line and Voith, 1986). However in the recent years this belief has been questioned as there is no evidence that dogs are motivated or driven by the desire to be "dominant" (De Keuster and Hildegard 2009) even in a competitive context, although some dogs may be more competitive than others. The likelihood that a dog tends to protect resources more than others can be determined by the subjective value of the resource given by a particular individual (Shepherd, 2002) and not necessarily to a hierarchical motivation. Also, it is likely that the response of the dog is determined to a large extent by prior experiences and contexts (Bradshaw et al., 2008). It is more likely that most cases of aggression towards family members are driven by a conflict between owners and dogs resulting from interactions the outcome of which cannot be predicted (Leuscher and Reisner, 2008).

Guy et al., (2001) found that dogs that had a history of having bitten a person were reported to be fearful of more stimuli than dogs without aggressive antecedents. In their study on English Cocker Spaniels, Podberseck and Serpell (1997) also observed that dogs presenting higher levels of aggressive behavior were more likely to react to loud or high-pitched noises than less aggressive dogs. Interestingly, we did not found any relation between non social fears or phobias and aggression towards owners; however, caution should be taken when comparing the results of these studies with ours. In the first place, they included dogs that had already bit (Guy et al., 2001) and

with higher levels of aggressive behavior (Podberseck and Serpell, 1997) while we included all dogs presented for aggression towards the owners, suggesting that the relationship between aggression and fear is likely to be significant in the most severe cases. Second, we should consider again that the control group we had included dogs with other behavioral problems. Although we do not collected information about the behavioral problems control dogs had, apart from the fact that they were not aggressive towards the owners, it is possible that many of them suffered from fear related problems, thus masking possible differences between the two groups.

When evaluating the postures of the dogs, we observed that ambivalent signals were the most frequently observed during the aggressive events followed by defensive postures and lastly by offensive ones. This fact reinforces the idea that aggressive behaviour is driven by different underlying motivations. Ambivalent signals have been described as a mixture of body signals arising from internal conflict (Beaver, 2009). In fact, in aggressive dogs, conflict behaviours indicate some degree of stress and uncertainty (Leuscher and Reisner, 2008). In captive wolves, ambivalence seems to be a very common behavioural expression, not related to any social status and perhaps reflecting a state of social stress (Fatjó et al., 2007). Social stress in dogs living with their owners is likely to be the consequence of inconsistency of the interactions between them and the application of inappropriate training methods such as punishment. As we pointed out in the introduction, inconsistent interactions can make the environment unpredictable for the dog thus leading to fear and aggression in the animal (Arhant et al., 2010).

Although in our study males were overrepresented in the group of aggressive dogs, the differences between males and females were not significant. This is contrary to previous studies that found a higher incidence of aggressive behavior in males than in females (Amat et al., 2009; Borchelt, 1983; Fatjó et al., 2007; Reisner et al., 2005). It should be considered that the effect of testosterone has been linked especially to an offensive type of aggression (Nelson, 2005) and in our study the majority of dogs

presented ambivalent or defensive signals. It has to be taken into account however that in the study by Fatjó et al., 2007 not all dogs displayed an offensive aggressive posture during the attacks and males were also found to be more prone to show aggression than females. The authors of that study postulated that the influence of sex hormones can be related to specific dimensions of aggressive behavior, like reactivity and impulsiveness since testosterone seems to reduce serotonin turnover in the central nervous system (Nelson and Chiavegatto, 2001). Low levels of serotonin have been associated with impulsiveness in aggressive dogs (Amat et al., 2013; Reisner et al., 1996). The possibility that the absence of significant differences between males and females in our study is due to a small sample size cannot be ruled out.

No differences were found between the frequencies of walks nor between the total amount of time spend walking every day in the aggressive and control groups. Jagoe and Serpell (1996) observed that dogs that exercised on a regular basis had lower prevalences of aggression toward the owners than less active dogs, suggesting that a more interactive relationship with the dog would be beneficial in preventing aggression. On the other hand, Podberseck and Serpell (1997) found that dogs with higher levels of aggression were more likely to be given less time for walks or exercise than less aggressive dogs and they proposed that this finding was a consequence of the fact that higher aggressive dogs were more likely to pull on the lead when walked. Increasing the amount of exercise is recommended as part of the treatment of aggressive dogs because it seems to reduce the level of anxiety (Leuscher and Reisner, 2008). Also, physical exercise was found to increase serotonin levels at least in laboratory rodents (Chaouloff, 1997). The lack of significant differences we found may be related to the fact that in general both groups spend a reasonable amount of time exercising every day.

Conclusions

The results of this study provide evidence that early adoption, sharing treats from the table and having a painful condition are causal factors significantly related to aggressive behaviour towards owners. The description of the aggressive events suggest that many dogs showing aggression towards owners may be suffering from social stress that could be a consequence of inconsistent interactions with the owners. These findings provide an interesting insight into some of the factors related to canine aggression towards family members and may help to develop more effective preventive and treatment strategies.

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DISCUSION GENERAL

Primer capítulo

Diferencias individuales en conducta agresiva detectadas mediante el cuestionario C-BARQ

Mediante el cuestionario C-BARQ, pudimos recoger información acerca de la conducta de los perros que acudieron a la consulta de Etología Clínica en un período determinado de tiempo. Los 13 factores extraídos de las preguntas del cuestionario C-BARQ fueron correlacionados entre sí con el objetivo de identificar características conductuales relacionadas con la conducta agresiva y de esta manera ayudarnos a comprender ciertas diferencias individuales en dicha conducta.

Relación entre "agresividad hacia personas de la familia" y" conductas de apego y demanda de atención"

Por un lado, hemos identificado una correlación entre los factores "agresividad hacia los propietarios" y "apego y demanda de atención" hacia los mismos. Este hallazgo resulta particularmente interesante ya que podría ser indicativo de un estado de ansiedad en perros que presentan agresividad hacia sus dueños. La relación entre agresividad hacia miembros de la familia y ansiedad en perros ha sido sugerida por otros especialistas. Así, por ejemplo, Reisner (2003) propone que la ansiedad juega un papel importante en la génesis de la agresividad y que de hecho, la mayoría de los perros con problemas de agresividad parecen motivados a morder por ansiedad o miedo.

Una vez sugerida la relación entre la agresividad hacia personas de la familia y la ansiedad en el perro, automáticamente surge la cuestión de cuáles serían los factores que desencadenan un problema de ansiedad en el perro. Ciertos factores como la genética y experiencias de la vida temprana pueden predisponer a los individuos a desarrollar problemas relacionados con la ansiedad (Kibusui et al., 2004) y agresividad

(Mertens, 2002). Además, el aprendizaje recibido por el animal durante su vida adulta puede determinar la aparición de este problema. Algunas prácticas de manejo por parte de los propietarios, por ejemplo, se han sugerido como posibles factores causales de la ansiedad y agresividad en perros. Así, muchos dueños intentan corregir ciertas conductas mediante la utilización de castigos. El uso de castigos se ha asociado con la aparición de conductas indeseables como la agresividad hacia personas de la familia (Blackwell et al., 2008). La aplicación de castigos puede desencadenar miedo en el animal y a través de un proceso de condicionamiento clásico puede desarrollarse un problema de ansiedad en anticipación al estímulo que le provoca miedo. La ansiedad también puede surgir por la falta de predictibilidad en el ambiente (Reisner, 2003). Así, el manejo poco estructurado y por lo tanto inconsistente e impredecible, puede generar estrés, ansiedad, conflicto y agresividad en el perro. La incapacidad del perro para predecir lo que ocurrirá puede ser una de las principales causas de conflicto entre el propietario y el animal, ya que este último no sabe qué respuesta esperar del propietario, ni tampoco cuál es la conducta más apropiada para la situación (Leuscher y Reisner, 2008).

Relación entre "agresividad hacia personas de la familia" y "excitabilidad" y "grado de energía"

El factor "agresividad hacia personas de la familia" también mostró correlación con los factores "excitabilidad" y "grado de energía". Un perro que es excitable tiene más probabilidades de ser agresivo (o de mostrar cualquier otro problema de comportamiento) (Leuscher y Reisner, 2008). Guy et al. (2001) de hecho observaron que los perros con agresividad hacia los dueños tendían a ser más excitables que los perros que no eran agresivos. La relación entre agresividad y excitabilidad podría explicarse en dos direcciones, es decir que la excitabilidad sea una causa o una consecuencia. Así, los perros muy excitables, podrían ser más propensos a la frustración y agresividad. Por otro lado, la excitabilidad podría ser una manifestación de ansiedad.

Relación entre "agresividad hacia personas desconocidas" y "agresividad hacia perros desconocidos" y "costumbre de cazar"

Algunos autores han sugerido que ciertas diferencias de comportamiento entre razas son consecuencia, al menos en parte, de diferencias en el grado de neotenia (Coppinger et al., 1997; Frank y Frank, 1982). El concepto de neotenia hace referencia al proceso evolutivo mediante el cual se produce una retención de los caracteres juveniles en el animal adulto como consecuencia de un enlentecimiento del desarrollo (Coppinger y Schneider, 1995). Según esta hipótesis, los perros adultos se comportarían en gran medida como lobos juveniles (Lindsay, 2000). La neotenia constituiría un requisito fundamental para la domesticación, dado que sería indispensable para facilitar la convivencia de los perros con las personas. Así, como resultado del proceso de neotenia, el perro adulto retiene características juveniles que son deseables en el entorno doméstico, tales como una escasa agresividad, una mayor dependencia del propietario, una mayor inclinación al juego, etc. (Manteca, 2003). Se observa también que los perros establecen relaciones sociales con los humanos mucho más fácilmente que los lobos. Además, los perros son más amistosos en general hacia individuos desconocidos de su misma especie que los lobos, que a medida que crecen se muestran cada vez más intolerantes hacia los desconocidos no pertenecientes a su manada (Lindsay, 2000). Otra característica que ha resultado del proceso domesticación del perro es la atenuación del instinto predatorio en comparación con el lobo. Cuando observan una presa, los lobos adultos manifiestan una secuencia que incluye acecho, persecución, captura y muerte. Si se enfrentan a la misma presa, la mayoría de los perros no hacen más que jugar o provocar al animal, conductas que equivalen a las mostradas por los cachorros de lobo.

Tal como mencionamos antes, se ha propuesto que la variabilidad que existe entre razas podría explicarse, al menos en parte, por diferentes grados de neotenia. Este hecho podría explicar, por ejemplo, las diferencias entre perros seleccionados para guiar al ganado, como el Border Collie, y los seleccionados para proteger al ganado como el Pastor de Maremma (Coppinger y Schneider, 1995). Los primeros exhiben

secuencias predatorias como perseguir, acechar e incluso atrapar a los animales, aunque sin llegar a hacerles daño, mientras que los segundos carecen de dichas conductas de depredación y se considerarían por lo tanto más neoténicos.

En nuestro trabajo, hemos encontrado una correlación entre los factores costumbre de cazar y agresividad hacia perros y personas desconocidos. Teniendo en cuenta la hipótesis propuesta por Coppinger et al. (1997) acerca de las diferencias en el grado de neotenia, podríamos suponer que las correlaciones encontradas en nuestro trabajo corresponden a un grupo de animales que se caracteriza por un menor grado de neotenia, ya que presentan una marcada conducta de depredación y a su vez manifiestan agresividad hacia individuos desconocidos –tanto hacia perros como hacia personas-. Evidentemente, esta explicación es sólo una hipótesis y se necesitan más estudios para confirmarla. Sería fundamental, por ejemplo, incrementar el número total de animales y el número de razas.

Segundo y tercer capítulo

Efecto de la manipulación neonatal sobre las diferencias individuales en la respuesta de estrés en cachorros

En nuestro estudio, observamos diferencias significativas en la concentración de cortisol y temperatura rectal antes y después del OFT lo que indica que este test representa un estímulo realmente estresante para los cachorros y, por lo tanto, constituiría una herramienta apropiada para evaluar la respuesta fisiológica de estrés.

En estudios en roedores de laboratorio, se ha observado que los cachorros manipulados durante la etapa neonatal mostraban concentraciones de glucocorticoides más bajas cuando eran sometidos a una situación estresante a los 3 o 4 meses de vida que aquéllos que no habían sido manipuladas (Meerlo et al., 1999). En nuestro trabajo, no encontramos diferencias en la concentración salival de cortisol entre cachorros manipulados y no manipulados durante la etapa neonatal. Las razones

por las cuales no hemos encontrado diferencias entre animales manipulados y no manipulados pueden ser diversas. Una de las hipótesis que creemos más probable es que se trate de un "efecto techo", es decir, que una vez alcanzado cierto nivel de estimulación, el hecho de aumentar el grado de estimulación ya no provoca cambios significativos. Esto podría ocurrir en el caso de que las madres que participaron en el estudio hayan lamido lo suficiente a los cachorros y que, por lo tanto, el hecho de proporcionar una estimulación adicional no haya generado beneficios adicionales. No podemos descartar, además, que los cuidadores del establecimiento hayan manipulado a los cachorros control más de lo que les habíamos indicado. En el estudio realizado por Gazzano et al. (2008) se observó un efecto positivo de la manipulación neonatal en cachorros de perro sobre varios parámetros de conducta. En dicho estudio, cada uno de los tratamientos (cachorros manipulados y cachorros no manipulados) se subdividió en cachorros criados en criaderos y cachorros criados en un domicilio familiar. Los autores observaron que las diferencias encontradas entre cachorros manipulados y no manipulados eran más pronunciadas en los cachorros de criadero, cuyo ambiente suele ser más pobre en estimulación que el de un ambiente familiar, sugiriendo que la manipulación en cachorros que ya están sujetos a un nivel mayor de estimulación no sería tan efectiva. Este hallazgo estaría, al menos en parte, en concordancia con nuestra hipótesis en la que sugerimos un posible efecto techo.

No podemos descartar que los resultados hayan sido afectados por factores relacionados con la genética de los perros. En ratas, se ha observado que la manipulación neonatal no presenta ningún efecto en algunas líneas genéticas de ratas (Durand et al., 1998). Es posible que en perros ocurra un fenómeno similar y que el efecto de la manipulación sea efectivo sólo en algunas razas y en otras no. En el estudio de Gazzano et al. (2008) se utilizaron animales de 7 diferentes razas y es probable que esto explique, al menos en parte, el hecho de que ellos hayan encontrado algunas diferencias entre tratamientos y nosotros no.

Efecto de la camada sobre las diferencias individuales en la respuesta de estrés en cachorros

Si bien no encontramos diferencias entre cachorros manipulados y no manipulados, sí que encontramos diferencias significativas entre camadas de cachorros en las variables cortisol salival y temperatura corporal y en las diferencias individuales en conducta. Estas diferencias pueden explicarse por diferentes razones. Tal como mencionamos en la introducción general, la concentración salival de cortisol muestra una considerable variabilidad individual que puede enmascarar posibles diferencias entre los tratamientos experimentales. Uno de los factores que puede contribuir a explicar dicha variabilidad está relacionado con diferencias en conducta maternal. En roedores de laboratorio, se han observado diferencias naturales en conducta maternal y se ha comprobado que estas diferencias pueden provocar a su vez diferencias neuroendocrinas en la respuesta de estrés de las crías (Liu et al., 1997). Así, cuando llegan a adultas, las crías de madres que exhiben una conducta de lamido más pronunciada durante los 10 primeros días de vida, muestran una concentración plasmática de ACTH y corticosterona frente a una situación de estrés agudo inferior a la de las crías de madres que lamen menos. Además, las crías de hembras más maternales presentan un incremento de la expresión del ARN mensajero del receptor hipocampal para glucocorticoides, un incremento de la sensibilidad del mecanismo de retroalimentación que regula la síntesis de glucocorticoides y una disminución del ARN mensajero de la hormona hipotalámica liberadora de corticotropina (Liu et al., 1997). Aunque, no conocemos ningún trabajo que evalúe directamente las diferencias en conducta maternal durante el período neonatal en el perro, sí que se han descrito diferencias en la conducta maternal de la perra en etapas posteriores y dichas diferencias parecen tener efecto sobre varios aspectos de la conducta de los cachorros (Wilson, 1984). Es posible, además, que las diferencias en la conducta maternal de las perras sean consecuencia de diferencias en su temperamento, de modo que las perras más miedosas muestren una conducta maternal menos pronunciada en presencia de los cuidadores. En definitiva, nuestra hipótesis es que las diferencias entre perras en cuanto a su conducta maternal pueden haber sido lo suficientemente pronunciadas como para causar diferencias entre camadas y enmascarar además el posible efecto de

la manipulación neonatal. Es indudable que esta hipótesis debería confirmarse mediante el estudio directo de la conducta maternal de las perras. Además, en el diseño original del experimento estaba previsto repetir el OFT y evaluar la respuesta fisiológica de estrés de los cachorros a las 20 semanas. Sin embargo, esto no fue posible porque los cachorros abandonaron el establecimiento antes de completar el estudio. El hecho de evaluar a los cachorros a una edad más avanzada, podría habernos ayudado a evaluar la consistencia de los resultados.

Las diferencias individuales en la respuesta conductual entre camadas podrían estar influidas además por el grado de maduración de cada camada. Las diferencias en la madurez entre animales pueden afectar la fiabilidad de los resultados ya que los animales de diferentes edades podrían reaccionar a estímulos potencialmente peligrosos de diferentes maneras. En un estudio realizado con ciervos jóvenes, se observó que el comienzo de la respuesta de huida dependía más del desarrollo físico del animal que de la edad (Espmark y Langvant, 1985). Así, por ejemplo, cuando los ciervos eran perturbados por personas, los ciervos de menor peso permanecían en una conducta de inmovilidad por más tiempo que los que tenían mayor peso. Es probable que pequeñas diferencias en el grado de desarrollo de los perros de las diferentes camadas pueda haber influido en los resultados. El hecho de tener en cuenta la variable peso hubiera sido de ayuda para verificar esta hipótesis.

Hubiera sido interesante, además, poder evaluar a los cachorros a una mayor edad, sin embargo, como lo hemos comentado anteriormente, esto no fue posible. Al evaluar a los individuos a una mayor edad, podríamos corroborar si las diferencias se deben o no al grado de maduración y además, cuanto mayor sea la edad del cachorro, sería más factible poder predecir la conducta del animal cuando llegue a la edad adulta. Wilson y Sundgren (1998) evaluaron la conducta de cachorros de 8 semanas mediante un test, la compararon con la performance de mismos como perros de trabajo a una edad adulta y observaron que la conducta del animal adulto no podía predecirse tan pronto como a las 8 semanas de edad. Estos resultados no necesariamente se aplican a nuestro trabajo ya que la evaluación de la conducta se hizo de manera diferente, pero sugieren que podría suceder algo similar.

Efecto de la edad sobre la concentración sérica de serotonina

Hemos observado que los niveles de serotonina sérica son significativamente más bajos en cachorros de 12 semanas de edad que en cachorros de 8 semanas. Estos resultados, sin embargo, son preliminares ya que sólo tres camadas pudieron ser evaluadas a las 8 y 12 semanas y por lo tanto tenemos pocos animales. De todas maneras, este hallazgo coincide con varios estudios en seres humanos en los que se ha observado también que los niveles de serotonina decrecen en adultos en comparación con niños (Chungani et al., 1999; Goldman-Rakic y Brown, 1982). El hecho de que las concentraciones de serotonina sean más altas en individuos en crecimiento que en adultos se explica por el rol que tiene este neurotransmisor en la regulación del cerebro en crecimiento (Whitaker-Azmitia, 2001).

Diferencias individuales en la respuesta conductual de estrés

Tal como comentamos en la introducción general, el OFT permite evaluar las respuestas de miedo producidas por el aislamiento y un ambiente desconocido (Walsh y Cummins, 1976). El miedo es un estado emocional negativo causado por la percepción de un peligro potencial. La respuesta de miedo incluye cambios conductuales que intentan neutralizar los efectos del estímulo que genera miedo (Boissy, 1995). Las expresiones de miedo son variables y pueden incluir tanto la defensa activa (ataque, amenaza) como la evitación activa (huir, esconderse) o la inmovilidad (inhibición del movimiento) (Boissy, 1995). Esta variabilidad de respuestas dificulta muchas veces la interpretación del OFT. La respuesta conductual durante el OFT puede estar además influenciada por numerosos factores tales como la genética, las experiencias durante fases iniciales del desarrollo, la edad del animal y la experiencia previa en el propio OFT. Por todos estos factores -dificultad de interpretación y alta variabilidad- en nuestro trabajo decidimos hacer un estudio multidimensional de los datos por medio de un análisis factorial. Así, pudimos extraer dos factores que juntos explicaron el 46.11% del total de la varianza. El primer factor

(Deambulación) describe a un grupo de animales que comienzan a deambular rápidamente una vez iniciado el test, caminan de un lado a otro y apoyan las patas y/o saltan sobre las paredes del campo de observación. El segundo factor (Reactividad) describe a un grupo de individuos que comienzan a caminar mucho más lentamente una vez iniciado el test y permanecen mucho tiempo en el centro del campo de observación. Dentro de este grupo de cachorros se pueden distinguir individuos más o menos reactivos. En un estudio realizado en zorros, Harri et al., (1995) encontraron dos patrones conductuales similares. Si bien estos factores identifican dos patrones diferentes de respuesta frente a una situación de estrés, no nos permiten concluir qué animales se estresan más En efecto, el hecho de que los animales de un grupo sean aparentemente más activos y realicen conductas dirigidas hacia las paredes, que podrían ser interpretadas como intentos de buscar una salida, no necesariamente significa que estén más estresados que los cachorros del otro grupo. Por el contrario, creemos más prudente suponer que se trata sólo de diferencias en la forma en que los distintos animales reaccionan frente al estrés. En estudios posteriores sería interesante profundizar en la correlación que existe entre estas diferencias de comportamiento y determinadas variables fisiológicas. Además, sería recomendable aumentar el número de animales.

Cuarto capítulo

Factores causales relacionados con la agresividad canina hacia las personas

Relación entre la edad de adopción y agresividad hacia personas de la familia

Se encontró que los perros adoptados después de las 12 semanas de edad eran menos agresivos hacia las personas de la familia que los perros adoptados tanto antes de las 3 semanas de edad como entre la tercera y la doceava semana de vida. Esta diferencia podría ser debida, al menos en parte, a que los propietarios de los perros adoptados después de las 12 semanas utilizaban el castigo en menor medida que los otros

propietarios. La utilización del castigo como método de entrenamiento puede facilitar la conducta agresiva (Arhant et al., 2010; Blackwell et al., 2008; Tami et al., 2008). Es posible que las personas que decidan adoptar a un perro de más de 3 meses estén más informadas o estén más predispuestas a informarse acerca del comportamiento y educación de los perros que las personas que adoptan perros de menor edad.

Además, y en el caso particular de los perros adoptados antes de las 3 semanas de vida, no es sorprendente que sean más agresivos que los adoptados con mayor edad ya que el destete precoz se ha asociado al desarrollo de problemas de comportamiento tanto en perros como en otras especies (Pieratoni y Verga, 2007; Kibusui et al., 2004; Rochtliz, 2005). El contacto suficiente con la madre parece ser un factor fundamental para el desarrollo de la estabilidad emocional del cachorro.

En los libros de Etología Clínica, una recomendación clásica es que los cachorros sean adoptados antes de las 12 semanas para asegurar una buena socialización con otras especies y habituación a estímulos. Si bien esta recomendación sería claramente justificable para prevenir problemas relacionados con el miedo, no es necesariamente útil para prevenir problemas de agresividad hacia los propietarios. Además, si la socialización y habituación son realizadas correctamente por el criador, el hecho de permanecer con la madre hasta las 12 semanas no debería suponer un problema. De todas maneras, creemos prudente seguir recomendando la adopción en la mitad del período de socialización del perro –es decir a las 8 semanas aproximadamente- si no se pueden asegurar una correcta socialización y habituación, pero haciendo mucho hincapié en el asesoramiento y educación de los propietarios acerca de cómo educar correctamente a los perros ya que según nuestros resultados este parece ser un factor fundamental para prevenir problemas de agresividad hacia los propietarios.

Relación entre dar comida de la mesa y agresividad hacia personas de la familia

Hemos observado que los perros que reciben comida de la mesa son más agresivos hacia los propietarios que los que no la reciben. El hecho de dar comida de la mesa podría reflejar un manejo inconsistente por parte del dueño. La falta de consistencia

en las interacciones ha demostrado estar asociada a la conducta agresiva (Arhant et al., 2010). Esto sucede porque el animal no puede predecir qué conducta debe realizar para recibir un premio, lo que puede generar ansiedad y frustración y agresividad (Leuscher y Reisner, 2008). El hecho de que a los perros que han recibido sesiones de entrenamiento reciban menos premios de comida fuera de la hora de comer (Voith et al., 1992) refuerza en parte nuestra hipótesis ya que según otros trabajos, los perros entrenados presentan una menor prevalencia de problemas de agresividad que los perros no entrenados (Jagoe y Serpell, 1996). El entrenamiento sería beneficioso para prevenir la agresividad porque genera un ambiente controlable y predecible para el perro (Leuscher y Reisner, 2008). Sin embargo, cuando el entrenamiento no es adecuado (por ejemplo, cuando se utilizan castigos) puede ser contraproducente (Blackwell et al., 2008).

Otra explicación sería que los dueños de perros ya agresivos respondan a las demandas del perro en un intento por mejorar la relación con el mismo o porque le tienen miedo. En este caso, el hecho de dar comida de la mesa representaría una consecuencia más que una causa de la conducta agresiva del perro.

Relación entre la agresividad hacia personas de la familia y el dolor

Según nuestros resultados, los perros que sufren dolor son significativamente más agresivos hacia personas de la familia que los que no presentan dolor. La relación entre el dolor y la conducta agresiva puede explicarse desde varios puntos de vista que pueden estar muy relacionados. En primer lugar, es posible que la conducta agresiva de estos perros esté relacionada con una respuesta defensiva para evitar manipulaciones que previamente resultaron ser dolorosas para el animal. Por otro lado, el dolor genera una respuesta de estrés y se ha comprobado que las hormonas del estrés facilitan la expresión de la conducta agresiva en roedores de laboratorio (Kurk et al., 2004). En perros, se ha observado una mayor concentración plasmática de cortisol en perros agresivos en comparación con perros control (Rosado et al., 2010) lo que indicaría una hiperactividad del eje HPA en estos perros. El estrés crónico, además, puede provocar una disminución de la actividad serotoninérgica, lo que contribuiría a

incrementar la conducta agresiva (Mellor et al., 2000). Es probable, además, que los perros que sufren una patología dolorosa realicen menos ejercicio físico. El ejercicio podría ayudar a prevenir problemas de agresividad ya que la actividad física regular aumenta los niveles de serotonina (Chaouloff, 1997).

En nuestro trabajo, también observamos que los perros que sufren dolor son más impulsivos, es decir, dan menos señales de aviso antes de atacar que los perros que no sufren procesos dolorosos. Es posible que la impulsividad observada en perros agresivos con dolor sea consecuencia de un proceso de aprendizaje que permite al animal anticiparse a una manipulación potencialmente dolorosa. La impulsividad, además, puede resultar de una menor concentración de serotonina (Reisner et al., 1996; Wright et al., 2012).

Contextos en los que aparecen conductas agresivas y posturas corporales adoptadas por los perros con agresividad hacia personas de la familia

Cuando evaluamos el contexto en el que los perros mostraron agresividad, observamos que la mayoría de los perros de nuestro estudio lo hacían en un contexto competitivo, seguido de un contexto de frustración y un contexto defensivo. El objetivo principal de evaluar el contexto en el que los perros mostraban agresividad fue el de poder deducir la motivación del animal. Sin embargo, la interpretación de los resultados no es en absoluto fácil. Esto se debe, principalmente, a que es probable que un perro experimente diferentes motivaciones de forma simultánea. Esta coincidencia en el tiempo de varias motivaciones es más probable en perros que lleven cierto tiempo mostrando la conducta agresiva, de modo que la motivación inicial de la misma puede haber cambiado según la respuesta del propietario.

En cuanto a la postura de los perros de nuestro trabajo, la mayoría de los mismos presentaba una postura ambivalente durante los ataques. Este hallazgo coincide con los resultados Leuscher y Reisner, (2008). Clásicamente, la mayoría de los casos de agresividad canina hacia personas de la familia eran diagnosticados como Agresividad por Dominancia. El término “Agresividad por dominancia” hace referencia a un

conflicto jerárquico entre el perro y el propietario, de forma que el perro, que se considera dominante sobre el propietario, reacciona con agresividad al percibir un desafío a su posición social por parte de un individuo de menor rango (Cameron, 1997; Line y Voith, 1986). Sin embargo, recientemente esta teoría ha comenzado a ser cuestionada por diferentes motivos. En primer lugar, no existen evidencias suficientes que confirmen que el perro doméstico muestre habitualmente tendencia a ser dominante sobre las personas (Bradshaw et al., 2009; De Keuster y Hildegard, 2009). Además, tal como observamos en nuestro trabajo, la postura que muestran la mayoría de los perros agresivos hacia sus propietarios es ambivalente y este hecho no es compatible con la hipótesis de la dominancia Por último, muchos perros con este tipo de agresividad muestran signos de ansiedad o miedo (Lesucher y Reisner, 2008). Este hecho coincide con el hallazgo que hemos observado en el primer capítulo, donde encontramos una correlación entre agresividad hacia los dueños y conductas relacionadas con la ansiedad (apego y demanda de atención). En otros trabajos, además, se ha observado que perros que han mordido a personas presentaban también en muchos casos problemas de miedo (Guy et al., 2001) y que los perros con agresividad severa eran más propensos a reaccionar frente a ruidos fuertes. En definitiva, pues, todos estos hallazgos son inconsistentes con la aproximación clásica y cuestionan la validez del diagnóstico de Agresividad por dominancia. Tal como comentamos en el capítulo 1, los problemas de agresividad hacia personas de la familia se explican mejor cuando se considera que surgen como resultado de interacciones inconsistentes o mala aplicación de castigos que pueden generar ansiedad y frustración en perros susceptibles, predisponiéndolos a mostrar agresividad.

Desde el punto de vista práctico, este enfoque actual tiene una repercusión importante tanto para la prevención como para el tratamiento de los problemas de agresividad. El hecho de que por muchos años se haya considerado que los perros establecían una relación de dominancia y subordinación con el dueño, ha dado lugar a que surjan una serie de prácticas de manejo destinadas a “dominar” al perro, muchas de ellas contraproducentes y susceptibles de generar miedo y estrés en el animal, además de ser potencialmente peligrosas para los propietarios.

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CONCLUSIONES GENERALES

1. La agresividad del perro hacia las personas de la familia parece estar asociada en muchos casos a un problema de estrés crónico y ansiedad en el animal, que a su vez podría ser consecuencia de un manejo inconsistente por parte de los propietarios. Además, existe una asociación entre la agresividad del perro hacia personas desconocidas y el miedo.

2. Existe una asociación entre la conducta depredadora y la agresividad hacia otros perros y hacia personas desconocidas; dicha asociación podría ser consecuencia del efecto de la neotenia sobre estas conductas.

3. En nuestras condiciones experimentales, la manipulación neonatal no ha demostrado tener ningún efecto sobre las diferencias individuales en las respuestas fisiológicas y conductuales de estrés.

4. La concentración sérica de serotonina en cachorros disminuye con la edad.

5. Los perros adoptados después de las 12 semanas de vida son menos agresivos hacia personas de la familia que los perros adoptados antes de esa edad.

6. Los procesos patológicos que causan dolor no solo aumentan el riesgo de que el perro muestre comportamiento agresivo hacia sus propietarios, sino que también aumentan el riesgo de que el animal reduzca o elimine las señales de aviso antes del episodio de agresividad.