Ph.D. Theses

Behavioral and physiological characterization of context-dependent aggressive behavior in dogs

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Budapest
2009

Introduction

We know from literature that the level of cortisol can be assessed in dogs using blood (pl. Haverbeke et al., 2008; Hennessy et al., 1997; Kolevská et al., 2003), saliva (pl. Coppola et al., 2006; Dreschel and Granger, 2009; Jones and Josephs, 2006; Kobelt et al., 2003) samples in case of acute stress, while urine (pl. Beerda et al., 1996; Hiby et al., 2006; Rooney et al., 2007a) and hair samples were used (pl. Accorsi et al., 2008) to measure cortisol when chronic stress was examined. It was showed that the cortisol levels could be affected by the dogs' age (Goy-Thollot et al., 2007; Hennessy et al., 1998; Reul et al., 1991), gender (Garnier et al., 1990) and species (Hennessy et al., 1997).

The signs of acute and chronic stress could be observed in dogs as well. (Beerda et al., 1997). Acute stress was examined in the case of family-, shelter- and working dogs, while chronic stress was studied only in the case of shelter- and working dogs. They caused acute stress to dogs by using different scary objects (Beerda et al., 1998, 1999a; King et al., 2003) or situations (Beerda et al., 1998; Bergeron et al., 2002; Dreschel and Granger, 2005; Haubenhofer and Kirchengast, 2007), and to that the dogs responded with elevated cortisol levels, and by showing specific behavior signs of acute stress (paw lifting, snout licking and low posture). The effect of the leash and muzzle on dogs' behavior and cortisol levels was also studied (pl. Ogburn et al., 1998; Steiss et al., 2007). Regarding to chronic stress it is documented that working dogs (Batt et al., 2009; Haverbeke et al., 2008; Rooney et al., 2007a) and shelter dogs (Beerda et al., 1999a,b, 2000; Hennessy et al., 1997; Hiby et al., 2006; Stephen and Ledger, 2006) that were kept in kennels have higher levels of cortisol comparing that were not.

There are also reports that humans can affect the dogs' cortisol levels. The people's testosterone levels (Jones and Josephs, 2006), personality (Kotrschal et al., 2009), gender (Hennessy et al., 1997; Kotrschal et al., 2009), or simple presence (Tuber et al., 1996) and petting (Coppola et al., 2006; Hennessy et al., 1998; Jones and Josephs, 2006) can affect the dogs' behavior and cortisol levels. This effect can diminish the stress (Coppola et al., 2006; Hennessy et al., 1997, 1998; Tuber et al., 1996), but also can elevate the cortisol levels of dogs (Jones and Josephs, 2006).

Main aims

We can conclude from data cited earlier that these studies did not examine closely the possible stress situations during social interactions, and their behavior and hormonal characterization is missing. Considering these facts, our aim was to examine the connections between behavioral signs generated during stress and social interactions in different situations in case of policemen and their dogs. We attained our aims through four interrelated experiments, each of them examined one interaction type between man and dog.

(**1**) In our first experiment (**Separation**) we planned to analyze the stress level initiated by separation. We wanted to examine how the dogs' behavior and cortisol levels change in a situation where the dog was separated from its owner.

We were curious to find out that *(a) in the well-trained police dogs the separation from handler would cause stress or not* and *(b) is there any relationship between change in cortisol levels and behavior?*

We hypothesized that the police dogs would be stressed by the separation from the handler, and their cortisol levels would elevate along with showing behavior typical associated with acute stress.

(**2**) In our second experiment (**Threatening approach**) we exposed the dogs to a threatening situation. In this situation we again looked for changes in behavior and hormonal levels of dogs.

In this case we were eager to get answers for the following questions: *(a) Is there any difference in behavior ('coping styles') after a social challenge on individual level in the case of police dogs? (b) Whether social stimulation triggered by the aggressor would affect the final cortisol levels of dogs (c) Is there a sort of connection between behavior ('coping styles') showed during the test and cortisol levels?*

We hypothesized that the police dogs could be characterized with a type of stress coping styles as well, which could be influenced by the age. The dogs would react to the stress generated by the aggressor with elevated cortisol levels, and the different coping styles would show characteristic hormonal levels.

(**3**) In the third experiment (**Play situation**) we not only examined the behavior of dogs playing with their handlers, but we analyzed the handler's behavior towards their dogs as well.

We wanted to know, that *(a) is the handler's behavior will affect the dogs' behavior, and (b) Is there a connection between the dogs' cortisol levels and behavior showed during the play?*

We expected to see a decrease in dogs' cortisol levels after a friendly encounter with the handler, and an increase in case of disciplining situation. On the other hand the cortisol levels of dogs that liked to play would decrease.

(**4**) In the last experiment (**Exam**) the dog and its handler participated in a final official exam, where the results were highly dependent on their collaboration.

Our questions were: *(a) Does the challenging situation will affect the hormone levels of policemen (testosterone- and cortisol levels) and their behavior towards the dog? (b) Is the profession of handler would influence the hormonal levels and behavior response indicated during the exam? (c) Would be stressful this situation to the dogs? (d) How the hormonal and behavioral changes in human will affect dogs' behavior and cortisol levels?*

We hypothesized that this situation will be stressful to the policemen, and their initial testosterone levels will have an effect on their behavior. Handlers with high basal testosterone levels will adopt a more dominant behavior towards their dogs, which in turn will increase the cortisol levels in dogs. The dogs will show typical behavior signs of acute stress. On the other hand handlers with normal or lower basal testosterone levels will behave friendly with their dogs and the dogs' cortisol levels will not change drastically. Furthermore the policemen's (patrol) basal testosterone levels will be higher than in border guard, and during the exam they will behave accordingly.

Materials and Methods

In four different situations mentioned earlier (separation, threatening approach, play situation, exam) we tested 179 policemen and their dogs, which they belonged to two different work groups: patrol dogs, border guard dogs. However in the final analysis we included only male German Shepherd dogs (N=123). The experiments were carried out between 2005 and 2007 at the Hungarian National Police Training School for Police Dog Handlers (Dunakeszi, Hungary). Besides examining the behavior of policemen, we also measured their cortisol and testosterone levels. In case of dogs we were able to test only the cortisol levels. Saliva samples were collected from handlers and dogs before, and 20 minutes after the end of the testing. The handlers spat into the numbered Eppendorf tubes. The dogs' saliva was collected with cotton swabs by the handlers. For long term storage the saliva samples were kept in a deep freezer (-80

ºC). Just before the analysis the tubes were warmed up to room temperature. The saliva was removed from the cotton swabs by centrifugation (3000 rpm for 15 min) using special centrifuge tubes with filters (Corning Spin-X; Sigma-Aldrich Ltd., Budapest, Hungary). After separation the saliva samples were analyzed for cortisol and testosterone concentrations using a highly sensitive enzyme immunoassay kit from Salimetrics (State College, PA, USA).

(**1**) **Separation**. In this experiment we tested 36 dogs. The dogs were tight to the tree by the handler and left there for 5 minutes. Besides measuring the cortisol level, we also examined their behavior during the separation (sitting, standing, lying etc.), greeting the handler, orientation toward the handler, looking away and the vocalization (bark, yowl).

(**2**) **Threatening approach**. In our experiment we used a modified test of Vas et al. (2005). We measured 60 dogs' cortisol levels and their behavior. The dogs were tied up to the tree and they had to endure 3 consecutive threatening situation. During the first two rounds the dogs were alone. In the last situation the handlers stand beside their dogs. Along recording the dogs' behavior (see Separation) we also analyzed to their orientation toward the decoy and attack latency.

(**3**) **Playing situation**. 53 patrol- and 31 border guard dogs were tested. A method published by Rooney and Bradshaw (2002) was used to test the dogs. The dogs played with their handlers for 3 minutes. The handlers could choose between rag and playing rope to encourage the dogs to play. We tested not only the playing behavior of dogs (playfulness, motivation, willingness to retrieve, possessivity), but we measured the latency time of starting to play as well. Furthermore we also coded the behavior of the handlers towards their dogs (control commands, sound signal, praising, petting the head and body).

(**4**) **Exam situation**. 27 patrol- and 17 border guard dogs were tested during a final exam. We coded special attention to the dogs` behavior towards the handlers (re-grab, release sleeve, aspire to decoy, vocalization) and vice versa. The behavior of dogs and handlers were backed up with hormone levels measurements.

Results and Discussion

(**1**) **Separation**. As a response to the separation the dogs' cortisol levels increased. On the basis of dogs' behavior it seems that beside the obvious stress caused by the separation, we were able to measure also the different attachment types to the handlers. We concluded this on the basis of

two main factors describing dogs' behavior (exploration and reactivity), and by analyzing how the dogs greeted the handlers. During the test the dogs included in the first group explored the environment (but they did not look after the handler), or nuzzle and when the handler returned there were no obvious signs of greeting. The signs of stress were missing. On the basis of these results we can conclude that the dogs included in this group behaved similarly to human infants with insecure-avoidant attachment. They did not stress and when the parents returned they were indifferent to them. The dogs included into the second group behaved like babies with insecure-ambivalent attachment behavior. They were active during the whole test, and mainly interested in environment, but when the handlers returned they did not greet them. During the test period they often vocalized, barked and whined, what could be the signs of separation stress. Dogs belonged to the third group often laid during the test, and looked in the direction in which the handler left the scene. They did not show any sign of stress. However, we did not get significant difference regarding how the different groups greeted the handlers, but still this last group got the highest score. Regarding the cortisol levels we did not find significant difference neither inside the groups nor between the groups. But it is worth mention that we saw an elevated tendency in the case of the first two groups, but not in the third one. When we studied the first to groups together -keeping in mind that their behaviors were similar and the cortisol levels showed the same tendency- (groups 1 and 2 active/reactive, 3. group passive), we found that the cortisol levels was significantly increased. These data suggest that in our case the dogs that can be described by unsecure attachment behavior type after separation from the handlers will respond with elevated levels of cortisol. On the other hand on dogs with secure attachment behavior type the separation from handler did not cause stress.

(**2**) **Threatening approach**. The police dogs on individual levels responded differently to the threatening situation. These were apparent in behavior and cortisol levels as well. The dogs applied different coping styles. The first group of dogs used a passive strategy to deal the stress ('Passive' group), which can be characterized with long attack latency, and reacted with passive and submissive behavior to the approaching decoy. Their cortisol level increased moderately. The cortisol levels in dogs belonged to the second group remained unchanged, and they showed high physical activity during the test, with short attack latency combined with aggression. So, these dogs can be characterized with proactive coping styles against social stress ('Proactive' group). The behavior of the third group of dogs differed completely from the previous two. Their

behavior showed many ambivalent sign: while the decoy was away they were active, barked, but as soon as he started to approach they started to shown the signs of acute stress (paw lifting and licking etc.). All these signs suggested that they were not able to choose a specific coping style against this type of social stress ('Ambivalent' group). This group had an elevated cortisol level, and it seems that this behavior type is the most inefficient and dangerous. Because they do not know how to handle this situation they are exposed to higher stress level than the two other groups: proactive and reactive.

(**3**) **Play situation**. The results suggest that the behavior of handler towards the dog had a greater effect on dogs than the playing situation itself. During the play sessions police officers were mainly disciplining their dogs, and this behavior elevated the dogs' cortisol levels. The border guards were truly playing with their dogs (including affiliative and affectionate behavior), and cortisol levels of border guard dogs decreased. Further analysis of the data revealed that changes in cortisol levels in dogs initiated by the handler's behavior were dependent on dogs' age and living conditions. The old patrol dogs were more affected by the dominant behavior of the handler than the younger one. The affiliative behavior of the handler had a more obvious reposing effect on younger border guard dogs than on the older one. The initial cortisol level of border guard dogs kept at home was higher than the police dogs used to the kennels.

(**4**) **Exam**. On the basis of basal testosterone levels the patrols were classified in two groups: high basal testosterone level (HBT) and low basal testosterone levels (LBT). By comparing the changes in hormonal levels of the three groups during the exam, it is turned out that in the case of HBT patrols the cortisol levels remained high along with significant decrease of testosterone. Both hormone levels showed an increasing tendency in case of LBT patrols, while in case of border guards the cortisol showed a decreasing tendency along with significant increase of testosterone levels. After analyzing the behavior of the handlers we have found an interesting thing. The border guards differed from patrols not only in their hormonal levels, but they were different in their behavior towards the dog as well. The HBT patrols praised their dogs more often; the LBT patrols usually disciplined their dogs. During the exam the border guards pated their dogs. The level of cortisol raised in all three groups independently from handler's behavior. We did not find behavior differences either. The highest official score was achieved by the LBT dogs, and the lower one by the border guard dogs.

The different situations and social challenges sometimes affected more the handlers and other times the dogs. We were able to identify different strategies used by both human and dogs to deal the stress, which we characterized using behavioral and hormonal markers. Differences in behavioral styles used by dogs were most obvious in the threatening situation, but we found interesting odds during the separation tests as well. To the handlers the most challenging social situation was the exam itself. The strategies adopted to deal the stress were influenced by many factors in humans and dogs too. In humans the testosterone level played the most important role. In the case of dogs the temperament, age, previous experience and living conditions affected the outcome of the response. Humans were able to affect dogs' behavior in a stressful situation, which was highly obvious in playing interactions.

New scientific results

1). We found that the dogs' behavioral responses during the separation show high similarity to the three attachment types described in human infants: secure, insecure-avoidant and insecure-ambivalent. Furthermore, we showed that the different separation behavioral types manifested in changing tendency of cortisol levels as well. The cortisol levels showed elevated tendency in those dogs that did not use the owner as a secure base, while in case of dogs looking for protection remained unchanged.

2). We described for the first time that different coping styles exist in police dogs. Beside the two existing coping styles (proactive and reactive or passive) we discovered a third one (ambivalent). The latter one differs from the two others in both behavior and hormonal response.

3). We reported that the playing situation alone was not enough to decrease the stress. Beside the playing situation, the handler behavior and the type of interaction with the dog influenced the dogs' cortisol levels. The owner's dominant behavior caused the cortisol levels to rise, while the affiliative behavior had a negative effect on cortisol levels.

4). We showed that in exam situations the cortisol levels elevated in dogs. The behavior of policemen during the exam towards their dogs was influenced by their baseline testosterone levels and subjective social status. On the basis of our 'Compensation hypothesis' policemen with low baseline testosterone levels tried to achieve a higher subjective social status by applying a more dominant behavior toward their dogs.

5). Finally, all of our experimental results point in one direction, namely that in older dogs the challenge situations evoke higher stress. Behavior and hormonal measurements in threatening approach test could be used to assess the welfare of the older dogs.

References

Accorsi, P.A., Carloni, E., Valsecchi, P., Viggiani, R., Gamberoni, M., Tamanini, C., Seren, E., 2008. Cortisol determination in hair and faeces from domestic cats and dogs. *General and Comparative Endocrinology* 155, 398-402.

Batt, L.S., Batt, M.S., Baguley, J.A., McGreevy, P.D., 2009. The relationships between motor lateralization, salivary cortisol concentrations and behavior in dogs. *Journal of Veterinary Behavior: Clinical Applications and Research* 4, 216-222.

Beerda, B., Schilder, M.B.H., Janssen, N.S.C.R.M., Mol, J.A., 1996. The use of saliva cortisol, urinary cortisol, and catecholamine measurements for a noninvasive assessment of stress responses in dogs. *Hormones and Behavior* 30, 272-279.

Beerda, B., Schilder, M.B.H., van Hooff, J.A.R.A.M., de Vries, H.W., 1997. Manifestations of chronic and acute stress in dogs. *Applied Animal Behaviour Science* 52, 307-319.

Beerda, B., Schilder, M.B.H., van Hooff, J.A.R.A.M., de Vries, H.W., Mol, J.A., 1999a. Behavioural, saliva cortisol and heart rate responses to different types of stimuli in dogs. *Applied Animal Behaviour Science* 58, 365-381.

Beerda, B., Schilder, M.B.H., Bernadina, W., van Hooff, J.A.R.A.M., de Vries, H.W., Mol, J.A., 1999b. Chronic stress in dogs subjected to social and spatial restriction. I. Behavioral responses. *Physiology & Behavior* 66(2), 233-242.

Bergeron, R., Scott, S.L.S., Émond, J.P., Mercier, F., Cook, N.J., Schaefer, A.L., 2002. Physiology and behavior of dogs during air transport. *The Canadian Journal of Veterinary Research* 66, 211-216.

Coppola, C.L., Grandin, T., Enns, R.M., 2006. Human interaction and cortisol: Can human contact reduce stress for shelter dog? *Physiology & Behavior* 87, 537-541.

Dreschel, N.A., Granger, D.A., 2005. Physiological and behavioral reactivity to stress in thunderstorm-phobic dogs and their caregivers. *Applied Animal Behaviour Science* 95, 153-168.

Dreschel, N.A., Granger, D.A., 2009. Methods of collection for salivary cortisol measurement in dogs. *Hormones and Behavior* 55, 163-168.

Garnier, F., Benoit, E., Virat, M., Ochoa, R., Delatour, P., 1990. Adrenal cortical response in clinically normal dogs before and after adaptation to a housing environment. *Laboratory Animals* 24, 40-43.

Goy-Thollot, I., Decosne-Junot, C., Bonnet, J.-M., 2007. Influence of aging on adrenal responsiveness in a population of eleven healthy beagles. *Research in Veterinary Science* 82, 195-201.

Haubenhofer, D., Kirchengast, S., 2007. Dog handlers and dog's emotional and cortisol secretion responses associated with animal-assisted therapy sessions. *Society and Animals* 15, 127-150.

Haverbeke, A., **Diederich, C., Depiereux, E., Giffroy, J.M.**, 2008. Cortisol and behavioral responses of working dogs to environmental challenges. *Physiology & Behavior* 93, 59-67.

Hennessy, M.B., Davis, H.N., Williams, M.T., Mellott, C., Douglas, C.W., 1997. Plasma cortisol levels of dogs at a county animal shelter. *Physiology & Behavior* 62, 485-490.

Hennessy, M.B., Williams, M.T., Miller, D.D., Douglas, C.W., Voith, V.L., 1998. Influence of male and female petters on plasma cortisol and behaviour: can human interaction reduce the stress of dogs in a public animal shelter? *Applied Animal Behaviour Science* 61, 63-77.
Hiby, E.F., Rooney, N.J., Bradshaw, J.W.S., 2006. Behavioural and physiological responses of dogs entering re-homing kennels. *Physiology & Behavior* 89, 385-391.
Jones, A.C., Josephs, R.A., 2006. Hormonal interactions between man and the domestic dog. *Hormones and Behavior* 50, 393-400.
King, T., Hemsworth, P.H., Coleman, G.J., 2003. Fear of novel and startling stimuli in domestic dogs. *Applied Animal Behaviour Science* 82, 45-64.
Kobelt, A.J., Hemsworth, P.H., Barnett, J.L., Butler, K.L., 2003. Sources of sampling variation in saliva cortisol in dogs. *Research in Veterinary Science* 75, 157-161.
Kolevská, J., Brunclík, V., Svoboda, M., 2003. Circadian rhythm of cortisol secretion in dogs of different daily activities. *Acta. Vet. Brno* 72, 599-605.
Kotrschal, K., Schöberl, I., Bauer, B., Thibeaut, A.-M., Wedl, M., 2009. Dyadic relationships and operational performance of male and female owners and their male dogs. *Behavioural Processes* 81, 383-391.
Ogborn, P., **Crouse, S., Martin, F., Houpt, K**., 1998. Comparison of behavioral and physiological responses of dogs wearing two different types of collars. *Applied Animal Behaviour Science* 61, 133-142.
Reul, J.M.H.M., Rothuizen, J., de Kloet, E.R., 1991. Age-related changes is the dog hypothalamic-pituitary-adrenocortical system: Neuroendocrine activity and corticosteroid receptors. *J. Steroid. Biochem. Molec. Biol*. 40, 63-69.
Rooney, N.J., Bradshaw, J.W.S., 2002. An experimental study of the effects of play upon the dog-human relationship. *Applied Animal Behaviour Science* 75, 161-176.
Rooney, N.J., Gaines, S.A., Bradshaw, J.W.S., 2007. Behavioral and glucocorticoid responses of dogs (*Canis familiaris*) to kennelling: Investigating mitigation of stress by prior habituation. *Physiology & Behavior* 92, 847-854.
Steiss, J.E., Schaffer, C., Ahmad, H.A., Voith, V.L., 2007. Evaluation of plasma cortisol levels and behavior in dogs wearing bark control collars. *Applied Animal Behaviour Science* 106, 96-106.
Stephen, J.M., Ledger, R.A., 2006. A longitudinal evaluation of urinary cortisol in kenneled dogs, *Canis familiaris*. *Physiology & Behavior* 87, 911-916.
Tuber, D.S., Hennessy, M.B., Sanders, S., Miller, J.A., 1996. Behavioral and glucocorticoid responses of adult domestic dogs (*Canis familiaris*) to companionship and social separation. *Journal of Comparative Psychology* 110, 103-108.
Vas, J., Topál, J., Gácsi, M., Miklósi, Á., Csányi, V., 2005. A friend or enemy? Dogs' reaction to an unfamiliar person showing behavioural cues of threat and friendliness at different times. *Applied Animal Behaviour Science* 94, 99-115.

List of relevant publications

- **Papers published in peer reviewed journals**

Horváth, Zs., Igyártó, B.-Z., Magyar, A., Miklósi, Á. (2007). Three different coping styles in police dogs exposed to a short-term challenge. *Hormones and Behavior*, 52(5): 621-630. **IF 3,401**

Horváth, Zs., Dóka, A., Miklósi, Á. (2008). Affiliative and disciplinary behavior of human handlers playing with their dog affects cortisol concentrations in opposite directions. *Hormones and Behavior,* 54(1): 107-114. **IF 3,876**

- **Papers in Hungarian**

Horváth Zs., Sudár Zs., Miklósi Á. (2004). Rendőrkutyák agresszivitása. Engedelmes rendőrkutyák agresszivitása avagy agresszív rendőrkutyák engedelmessége. *Múzeumi Füzetek,* 13, Kolozsvár.

Horváth Zs., Miklósi Á., Kiss Sz. (2008). A munka típusának és a kutya jelenlétének hatása a rendőrök agresszivitásának mértékére. *Erdélyi Pszichológiai Szemle,* IX/1: 1-28.

- **Talks at conferences**

Horváth Zs., Miklósi Á. (2003). Rendőrkutyák agresszív magatartásának vizsgálata különböző viselkedési helyzetekben. VI. Erdélyi Tudományos Diákköri Konferencia, Kolozsvár, Románia; **III. díj**

Horváth Zs., Miklósi Á. (2004). Kutya-ember vizuális kommunikáció rendőrkutyáknál. V. Kolozsvári Biológus Napok, Kolozsvár, Románia

Horváth Zs., Miklósi Á. (2006). Hormonális és viselkedésbeli összefüggések vizsgálata rendőrkutyáknál. VII. Kolozsvári Biológus Napok, Kolozsvár, Románia

Horváth Zs., Miklósi Á. (2007). May seem like play, but is it? Ph.D. Tudományos Napok 2007, Budapest, Magyarország

Horváth Zs., Igyártó B.-Z., Magyar A., Miklósi Á. (2007). Rendőrkutyák stresszel való megküzdési stratégiái. Tavaszi Szél 2007, Budapest, Magyarország; **III. díj**

Horváth Zs., Miklósi Á. (2007). Ember és kutya hormonális interakciójának vizsgálata egy kihívásos helyzetben. X. Jubileumi Magyar Etológiai Kongresszus, Göd, Magyarország

- **Posters**

Sudár Zs., Horváth Zs. (2003). Rendőrkutyák agresszív magatartásának vizsgálata különböző viselkedési helyzetekben. ELTE TTK, Biológus Tudományos Diákkör poszter-konferenciája, Budapest, Magyarország

Horváth Zs., Miklósi Á. (2005). Old police dogs are more stressed by a threatening human. XXIX. International Ethological Conference, Budapest, Magyarország