CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

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MSc Thesis

THE USE OF SPECIALLY TRAINED CANINES TO DISCRIMINATE INDIVIDUAL ODORS OF IDENTICAL TWINS

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Author's Declaration

I hereby declare that I am the sole author of this thesis and that I have used only results of my own research or sources that are stated in the bibliography.

Prague, April 30, 2008 …………………………………

Acknowledgments

Abstrakt

V minulosti již byly podniknuty pokusy zjistit zda mají monozygotická dvojčata, která jsou vlastně geneticky identická, také identický individuální pach. Celá řada dosud publikovaných studií naznačuje, že MHC (hlavní histokompatibilní komplex) je odpovědný za individuální pach. Tyto studie jsou v souladu s několika málo experimenty, které byly na identických dvojčatech provedeny za použití speciálně vycvičených psů. Psi použití při těchto experimentech nedokázali rozlišovat individuální pachy monozygotických dvojčat, pokud tato žila ve stejném prostředí a pokud jejich pachy byly psům předloženy odděleně.

Při svém výzkumu jsem využil 10 speciálně vycvičených psů plemene německý ovčák, zařazených na třech různých krajských správách Policie České republiky. Psi srovnávali pachy dvou párů monozygotických a dvou párů dizygotických dvojčat ve věku 5, 7, respektive 8 a 13 let, která žila ve stejném prostředí a jedla stejnou stravu. Pachy byly odebírány do čtverců speciální bavlněné tkaniny, které byly uchovávány ve sklenicích se šroubovými uzávěry. Při odběru byly zachovávány předpisy a metody platné u Policie České republiky pro odběr pachových stop v souvislosti s trestným činem. Psovodi nebyli informováni o detailech experimentu a nevěděli o předpokládaných výsledcích identifikace. V průběhu pachové identifikace byly striktně dodržovány postupy platné pro ztotožňování pachů v případě kriminálního vyšetřování. Jeden pach byl vždy použit jako načichávací a druhý, porovnávaný pach, byl umístěn do řady mezi 6 klamných pachů. Jako klamné pachy byly použity pachy dětí obou pohlaví, podobného věku jako pachy dvojčat. Všichni psovodi o provedené pachové identifikaci sepsali protokol stejně jako v případě skutečné trestní věci. Tento protokol pak podepsali. Psi porovnávali pachy jednotlivých dvojčat a rovněž pak dva pachy odebrané od téhož dvojčete. Tím bylo prokázáno, že psi jsou skutečně schopni identifikaci pachů provádět a také tím byla prokázána přítomnost pachů ve sklenicích.

Všichni psi dokázali rozlišit pachy jak monozygotických, tak i dizygotických dvojčat, přestože tyto pachy byly psům předloženy odděleně tzn., že pes minul pach dvojčete v řadě jiných pachů aniž by ho označil. Všichni psi rovněž dokázali ve všech případech ztotožnit dva pachy odebrané od téhož dvojčete.

Má zjištění ukazují, že speciálně vycvičení psi dokáží rozlišovat individuální pachy jednovaječných dvojčat, přestože tato žijí ve stejném prostředí a jedí stejnou potravu a to i když jsou tyto pachy psům předloženy odděleně a nikoliv těsně vedle sebe. Výsledky dosažené v mé studii se odlišují od předchozích studií pravděpodobně v důsledku odlišné metodiky výcviku.

Klíčová slova: monozygotická dvojčata, pachová identifikace, psi

Abstract

There have already been attempts to establish whether human monozygotic twins that are genetically identical have also identical individual scents. Many studies have been published suggesting that it is MHC (major histocompatibility complex) that is responsible for individual scent. These studies are in accord with a few experiments that have been done so far on identical twins using specially trained dogs. Dogs used in the experiments were not able to distinguish individual scents of monozygotic twins living in the same environments if the scents were presented to them separately.

In my research I used 10 specially trained police German shepherds of 3 Czech Republic Police Regional Headquarters. The dogs were supposed to match scents of two couples of identical and two couples on nonidentical twins at the age of 5,7, respectively 8, and 13 years, living in the same environments and eating the same food. Scents were collected on cotton squares that were stored in glass jars with twist-off lids. The regulations valid for the collecting evidence at crime scenes were observed. During the scent identification line ups all canine officers followed strictly the scent identification protocol used by the Czech Republic Police during real criminal investigations. Handlers were not aware of the experiment details and did not know anything about expected results. One scent used as a smeller was matched with a target scent that was placed in a line of 7 glass jars in total. Scents of children of similar age were used as distractors. All canine officers wrote and signed reports on each single line up. This procedure was carried out in exactly the same manner as on a real life criminal case. In the line ups dogs matched a scent of one twin with the other as well as two scents collected from every single individual to prove their efficacy as well as the presence of the scent in glass jars.

All dogs used in the experiment distinguished scents of identical as well as nonidentical twins despite the fact that the scents were presented separately to them i.e. they passed the jars in the lines without alerting to them. All dogs also matched positively two scents collected from the same individuals.

My findings indicate that specially trained dogs are able to distinguish individual scents of identical twins despite the fact that they live in the same environment and eat the same food even if the scents are not presented to them simultaneously. Different results in comparison with earlier studies might have been caused by the different training approaches. It is also possible that even monozygotic twin children that live in the same environment and eat the same food produce after all individual scents that differ enough so that properly trained dogs are able to distinguish them.

Keywords: monozygotic twins, scent identification, canines,

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List of Abbreviations

DZ	Dizygotic
GCH	Gas Chromatography
HLA	Human Leukocyte Antigen
HQ	Headquarters
IED	Improvised Explosive Device
MHC	Major Histocompatibility Complex
MS	Mass Spectrometry
MZ	Monozygotic
SIC	Scent Identification Canine
SPME	Solid Phase Micro Extraction
US	Ultra Sound
ZPP	Závazný pokyn policejního prezidenta (Police President Direction)

1. Introduction

Scent identification line-up performed by trained dogs is a method used by some European and American law enforcement agencies e.g. in the Czech Republic (Teryngel, 2002; Kloubek, 2008), Poland, Russia, Hungary (Szinak, 1985; Settle et al., 1994), Denmark, and Netherland (Kaldenbach, 1998; Stockham et al., 2004b) however it has not gained widespread acceptance in the United States, mainly due to the lack of scientific studies demonstrating the reliability of such method (Curran et al., 2005) . The line-ups are performed in accordance with different training principles and forensic regulations however basically human scent left by a perpetrator at the crime scene is later matched with the odor sample taken from the detained suspect (Kloubek, 2003). In the Czech Republic the line-ups are performed in accordance with very strict regulations and dogs must perform absolutely flawlessly to be certified as the results of the line-ups are admitted by the Czech Courts of Law as circumstantial evidence (Kloubek, 2002). Performance of these dogs, have many times been challenged by lawyers reasoning that the clues, dogs use during their work, are not known (Teryngel, 2002).

The superior olfactory acuity of dogs is well known as well as their ability to cross match human odors (Schoon and Debruin, 1994; Harvey and Harvey, 2003) however we still do not know for sure in spite of great efforts of researchers (Sommerville et al., 1990; Sommerville et al., 1994; Curran et al., 2005; Curran et al., 2007) what substances emitted by humans are crucial for the dogs so as they would be able to match odors of various individuals and whether the individual odors of people remain unchanged over the lifetime. There have been published papers suggesting, that individual human scent is genetically determined (Hepper 1988; Sommerville et al., 1990; Sommerville et al., 1994; Harvey et al., 2006).

In the Czech Republic Police the Direction of the Police President (ZPP č. 52/2007), that regulates canine scent identification, even uses presumption of genetic determination of human odor as a proven fact on which is the scent identification line-up procedure built. If this is true then identical twins should have the same scent pattern and police canines should not be able to discriminate their individual odors.

Surprisingly, only a few attempts have been made to solve this problem. The results showed that dogs are not able to distinguish individual odors of identical twins if presented to the dogs separately. Dogs were able to do so if they could sniff at both odors at the same time (Kalmus, 1955; Hepper, 1988; Harvey et al., 2006). In my research I have tried to answer the question using line up identification protocol utilized by the Czech Republic Police.

2. The Aim of the Thesis

The goal of my thesis was to establish whether specially trained police canines are able to discriminate individual odors of identical twins and thus contribute to the suggestions that individual odors of humans are genetically determined. Taking into account the above mentioned findings and numerous researches concluding that MHC could be responsible for the individual odor of vertebrates (Eggert et al., 1998; Schaefer et al., 2001; Singh, 2001; Thom et al., 2005; Willse et al., 2006), I hypothesized that the Czech Law Enforcement Canines would not be able to distinguish between juvenile identical twins that live in the same environments and eat the same food.

3. Literature review

3.1. Human scent

3.1.1. Human Individual Odor and Canines

It is widely known that human body is a rich source of odors and dogs are able to follow tracks laid down by humans as well as distinguish individuals one from another using human odor as a clue (Patterson, 1992; Syrotuck, 2000; Lindsay, 2000). Trained canines are often used by law enforcement agencies to match pieces of physical evidence or traces of odors collected at the crime scenes with presumed criminal perpetrators (Brisbin and Austad, 1991). Usually the odor taken from the suspect is presented as one in an array of different odors, and the canines make the match by alerting to the odor of the suspect and by ignoring the other odors (Schoon, 1998).

Implicit in this practice is the assumption that humans have unique individual odor or odor signature which can be identified regardless of the body part from which the odor comes. This assumption was repeatedly challenged by some authors concluding that dogs in fact are not able to reliably match scents collected from different parts of human body (Brisbin and Austad, 1991; Brisbin and Austad, 1993) thus contradicting previous findings of Kalmus (1955) that odors taken from the armpit could be matched with odors collected from hands. The experiments performed by Brisbin and Austand (1991, 1993) were later criticized as dogs used in their experiments were trained to discriminate only odor collected from the hands and not odors from other body parts (Sommerville et al., 1993). Apparently the canines were trained to discriminate the odor signature of hands but not a reliable specifying signature of the identity of the person per se (Lindsay, 2000).

Much more extensive study was later realized by Settle et al. (1994) proving that correctly trained canines are able reliably match scents collected from different parts of human body to the correct person. They stated: "Our results suggest that if dogs are selected well, sympathetically trained and entirely dedicated to scent discrimination in a well-managed unit they are likely to maintain a dependably high performance over long periods. Furthermore, they should be able to achieve high scores when given a choice of odor from six different people. Further investigations aimed at the dog's ability to work with the trace odors, distinguish people on the basis of age and gender, and reliably cross-match odors from different parts of the body need to be carried out. The dogs' olfactory sensitivity, selectivity, and memory, as well as its capacity for odor pattern recognition, are unlikely to be challenged by any artificial sensor in the foreseeable future" (Settle et al., 1994) .

The ability of dogs to detect human scent or follow it in the terrain is really extraordinary. In an experiment done more than 20 years ago, dogs were able to detect a single human fingerprint on a glass slides after 6 weeks indoor and 2 weeks outdoor however not all dogs were apparently able to perform with the same accuracy (King et al., 1964). Czech Police canine officers claim that their dogs are able to detect human scent on the articles retrieved from water streams, ponds, and swimming pools (Svoboda, 2007; Jehlik, 2007) or from discharged casings or parts of the exploded IEDs (Improvised explosive devices) (Bukvaj, 2007).

This is in accordance with American authors stating that their dogs were able to match odors collected from exploded pipe bombs loaded with low-explosive powders as well as with high-explosive materials (Stockham et al., 2004b) . The authors also reported that bloodhounds were able to match correctly scents collected from explosive device that was placed inside a 20-liter plastic bucked that was packed with dirt. The bucked was then suspended inside a 189-liter steel drum and detonated. Dogs also performed correctly on the scent traces secured from gasoline containers, one plastic and one metal that were placed on the ground and covered with one half liter of gasoline. The gasoline was ignited and allowed to burn for two minutes. The fire was then extinguished with water. In another paper (Stockham et al. 2004a) the same authors presented a case example from Philadelphia where human scent had been collected from an IED placed in a mailed package two days after the bomb squad rendered the device safe. After 2 days of car and pedestrian traffic bloodhounds were able to find a track of the perpetrator and follow it to his house.

Even more amazing is another case reported by Stockham et al. (2004a) from Washington DC that occurred in July 2002. A pipe bomb exploded inside a car and severely injured the driver. Shortly after the bombing, half-brother of the victim disappeared. His car was found in a Metro-parking garage with a suicide note. Seventeen days after the bombing the police dog matched a scent collected from the bomb fragments with a track on the sidewalk and followed it to the house of the perpetrator. The reported cases seem to be incredible however they are in accordance with a controlled experiment done in California. The bloodhounds could match scent samples, collected from volunteers, with a track and followed it correctly after 48 hrs even if the tracks were laid down on the hard surface like cement or asphalt and were crossed by 1 000 pedestrians and flushed down by torrential rain (Harvey and Harvey, 2003). Schoon (2005) tested the effect of the ageing of crime scene objects on the performance of scent identification canines in line-ups. She reported that the dogs performed faultlessly in matching odors collected on the same day however the results dropped to a lower level and were less reliable if the odor was 2 weeks to 6 months old. The dogs had not displayed systematic decrease in the identification and the author concluded that after some initial reduction of the scent intensity, more or less stable odor concentration was established in the

glass jars. The fact that some of the dogs did not show flawless results can probably be ascribed to insufficient quality of training or personality of the used canines.

The above mentioned experiments and real cases as well show that human scent can survive in the environments for quite a long time despite traffic, unfavorable weather conditions, and even on burned objects as well as on the IED fragments and discharged casings. Dogs also have the ability to determine direction on an odor trail left by a human. Using human scent gradient as a clue, dogs are able within only 3 footsteps to determine correct direction in which the person went (Hepper and Wells, 2005) .

3.1.2. Division of Human Odors

Some human odors are stable over time and those are genetically based. Others may vary with environmental or internal conditions (Curran et al., 2007). The above mentioned authors developed terminology dividing human odors into three basic categories.

- **Primary odor** – contains constituents that are stable over time regardless of diet or environmental factors.
- **Secondary odor** – contains constituents which are also endogenous but are influenced by diet and environmental factors.
- **Tertiary odor** – contains constituents that are present due to exogenous sources (i.e., lotions, soaps, perfumes, etc.)

Primary and secondary odors are released into the environment via skin glands in the form of sweat, oil, mucous, and other glandular secretions and also in the form of loose dead cells from the epidermis. Loose, dead cells that are also called rafts are constantly shed from the skin, respiratory tract and digestive tract. Thus the secretions of skin glands and rafts together with odors of toiletries and environmental scents create individual human odor (Syrotuck, 2000) however it seems that the SICs (scent identification canines) are able to identify humans regardless of secondary and tertiary odors as they are able to match an individual odors collected from the crime scenes with odors of suspected perpetrators collected after 5 or even more years (Bukvaj, 2007). I assume that after such a long time the secondary and tertiary odors are at least partly changed as the people can change their food preference, stop smoking, change toiletries, wear different attire, use drugs or medications, sustain various diseases etc.

3.1.3. Skin Glands

The human skin serves primarily for protection, temperature regulation and excretion. It is divided into two layers. The outer layer is called the epidermis and the inner layer is called the dermis. The inner layer contains most of the excretory glands and almost five million secretory glands. There are almost five million eccrine, apocrine, and sebaceous glands found in the dermis (Ramotowski, 2001) .

- **Apocrine glands** – situated at the base of hair follicles. In humans they are usually found in the area of axillae, areolae of the nipples, periumbilical, perianal, and the genital region. It seem that there are no functional apocrine sweat glands in prepubertal children and very old people (Syrotuck, 2000). The apocrine glands are from the phylogenic point of view older than eccrine glands. They produce chemical olfactory signals connected with basic biological functions (Trojan, 2003). The main constituents of apocrine sweat are protein, carbohydrates, and ammonia. Apocrine secretions are in fact odorless to the people. The strong and often offensive scent is produced by the bacterial flora residing in the apocrine regions that degrade apocrine secretions (Leyden et al., 1981; Bird and Gower, 1982). It has been proved that microbial activity is necessary for the formation of 5α-androstenone on the skin surface. If there are not bacteria present in the human axilla no odor occurs (Hurley and Slelley, 1960) Clear secretions of apocrine glands are in fact odorless until the proliferation of some gram-positive bacteria. The typical unpleasant odor of axilla is a result of the activity of coagulase-negative staphylocci and diphteroids (Shehadeh and Kligman, 1963; Labows et al., 1979).

- **Eccrine glands** – simple tubular glands situated on the whole body surface. They are most numerous at the sole of feet, palms and on the nape. They are real sweat glands and produce sweat that is a clear watery secretion. Eccrine glands play an important role in thermoregulation. Emotional stress may also stimulate their function. The eccrine gland secretion contains weak saline solution, heavy concentration of enzymes as well as nitrogenous compounds and other proteins (Syrotuck, 2000; Trojan, 2003). Ramotowski (2001) reports following composition of the eccrine sweat.

  Inorganic (major): sodium, potassium, calcium, iron, chloride, fluoride, bromide, iodine, bicarbonate, phosphate, sulfate, and ammonia.

  Inorganic (trace): magnesium, zinc, copper, cobalt, lead, manganese, molybdenum, tin, mercury.

Organic (general): amino acids, proteins, glucose, lactate, urea, pyruvate, creatinine, glycogen, uric acid, vitamins.

Organic (lipids): fatty acids, sterols.

Miscellaneous: enzymes, immunoglobulins.

Aside of the sweat glands there is another type of skin glands that seems to contribute to the individual human scent.

- **Sebaceous glands** – glands usually located in body regions where hair is present, including the face, and scalp (Ramotowski, 2001). They produce an oily secretion known as sebum. The sebum is released into adjacent hair follicles. From there it gets to the skin surface and oils the hairs as well as the outer keratinized layers of the skin thus helping to waterproof them. Sebum also plays a role in inhibiting growth of skin microorganisms (Sherwood et al. 2005). The sebaceous glands are most numerous on the face, scalp, upper trunk, and pubic area. It seems that all hair follicles are associated with the sebaceous glands however some of the sebaceous glands lead directly to the skin surface. The sebum contains predominantly fatty compounds (Syrotuck, 2000). Ramotowski (2001) reported following content of the sebaceous glands secretions.

  Organic (major): triglycerides 30-40%, free fatty acids 15-25% (saturated 50%, unsaturated 48%, polyunsaturated 2), wax esters 20-25%, squalene 10-12%, cholesterol 1-3%, cholesterol esters 2-3%.

  Organic (trace): aldehydes, ketones, amines, amides, alkanes, alkenes, alcohols, phospholipids, pyrroles, pyridines, piperidines, pyrazines, furans, haloalkanes, mercaptans, sulfides.

However secretions of the respiratory tract and genitourinary tract also harbor numerous microorganisms and thus take part in the human individual odor signature (Syrotuck, 2000) .

3.1.4. Human Odor Signature

The mechanisms by which human body creates individual odor are not well known however it is known that layer of the skin (epidermis) release dead skin cells, called rafts, into the environment. The human skin contains approximately 2 billion cells out of which 667 are released each second. The rafts together with the secretions of skin glands and other odor sources create a cloud of odor that drop to the ground remaining on the terrain (Syrotuck, 2000). There have been done extensive scientific researches in the efforts to establish compounds that are responsible for the human individual odor or odor signature. As a method for the extraction, separation and identification of the VOC (volatile organic compounds) emanated by the human body SPME GCH-MS (solid phase,

gas chromatography-mass spectrometry) analysis has been used. Analysis of axillary secretions showed presence of C_6-C_{10} straight chains, branched and unsaturated acids, and as the major odor causing compound was identified (E)-3-methyl-2-hexenoic acid. As other odor contributors were terminally unsaturated acids, 2-methyl C_6-C_{10} acids, and 4-ethyl C_5-C_{11} acids (Zeng et al., 1991; Zeng et al., 1996). Analysis of the secretions from the palms identified 63 compounds. The composition included acids, alcohols, aldehydes, hydrocarbons, esters, ketones, and nitrogen-containing compounds (Curran et al., 2007). In the search for the chemical compounds emitted by humans, that attract the yellow-fever mosquito, 277 compounds have been identified and proved to be the components of skin emanations. Comparison of the samples taken from different people showed qualitative similarities (Bernier et al., 1999; Bernier et al., 2000; Bernier et al., 2002). The fact that the more closely related individuals are the more similar individual odor they have, has been supported by an elegant experiment conducted by Ables et al. (2007). The authors based their work on the behavior of rats which investigate novel odors longer than familiar odors. The rats were habituated to a referent human odor using food and then the time of investigation was measured when the rats were presented with odors of humans of different degree of relatedness. The results showed that the rats investigated the odor the longer the less was the odor-donor related to the reference odor-donor and vise versa.

3.1.5. Major Histocompatibility Complex

The human major histocompatibility complex (MHC), that is highly polymorphic gene complex located at the chromosome 6, plays an essential role in the immunological recognition of self and nonself (Aguado et al., 1999; Santos et al., 2005) and aside of that function it has been recognized as a possible source of individual specific body odors (Eggert et al., 1998; Ferstl et al., 1998; 1998; Jacob et al., 2002; Yamazaki and Beauchamp, 2005). In humans MHC is often referred to as human leukocyte antigen (HLA) system (Robinson et al., 2001).

Mice and other rodents use clues associated with MHC to distinguish heterozygosity from homozygosity and thus avoid inbreeding or outbreeding of the individuals that would possess too different genomes (Penn and Potts, 1998; Yamazaki et al., 1976; Yamazaki et al., 1979; Eklund, 1997). Some studies have even concluded that MHC associated odor attractiveness may play a role also in human partner preferences and that women prefer odor of MHC- dissimilar men (Wedekind et al., 1995; Ober et al., 1997; Jacob et al., 2002). Heterozygosity at three key loci in the MHC was proved to be associated with facial attractiveness as faces of men that were heterozygous at those loci were judged more attractive by women than faces who were homozygous at one or more of the

loci (Roberts et al., 2005). In connection with the MHC Wedekind and Penn (2000) bibliographically reviewed literature to present hypotheses about the mechanisms that control odor production.

- MHC molecules are present directly in the urine and sweat and so they provide individual odors. They consider this hypothesis unlikely as MHC molecules are "large, involatile proteins and furthermore, denaturation of proteins in urine does not destroy the distinguishability of MHC-mediated odors by mice".

- MHC molecules bind to allele-specific subsets of peptides, and their volatile metabolites, such as carboxylic acids, may provide the odorants.

- MHC genes may influence individual odortypes by their impact on specific populations of microbial flora. In accordance with Wedekind and Penn (2000) this idea is inconsistent.

- MHC molecules change their conformation to bind volatiles, instead of peptides, and transport them to scent glands.

- MHC-bound peptides are metabolized and made volatile by microorganisms.

Despite of a considerable amount of work supporting evidence of the connection between MHC and individual odor we still do not know for sure if MHC is the only factor responsible for the unique, primary odor of each individual and whether the mechanisms that determine individual odor are the same in various species like human, mice, or rats. McDonald and Adamashvili (1998) reviewed scientific papers dealing with soluble HLA (sHLA) and citing more than 200 of them they concluded that: "The unique odor of each individual is determined by the HLA genotype of that individual. These odorotypes may be determined by sHLA or its degradation products". Among others they supported their conclusions by the experiment done by Kalmus (1955) on identical and non-identical twins with trained dogs.

Nonetheless the fact that the dogs had problems to distinguish identical twins but did not have problems to distinguish non-identical ones can simply mean that the odors of the identical twins may be similar. It is very well possible that the dogs were not trained properly to be able to distinguish them. On the contrary Thom et al. (2005) concentrated on the role of the MHC in scent communication concluding that: "There is incontrovertible evidence that MHC region is associated with type-specific odors in a number of species. Existing experimental paradigms have demonstrated that animals can discriminate between classes of MHC-associated odor with great acuity". However they continued: "At the moment, evidence for genuine individual recognition or discrimination, meaning the *learned discrimination among conspecific individuals* is lacking". Nevertheless the authors admitted that it was partly because their main experimental paradigms

were not used explicitly to address the question of individual recognition, but they were more like aimed on the testing if there are broader subgroups of MHC-associated odors and whether they are distinguishable.

3.2. Scent Identification Line-ups

The first who suggested collecting odors at the crime scene so as it could be later used for trained dogs was probably Austrian criminal jurist and examining magistrate Dr Hans Gross. In 1893 he published his book *Handbuch für Untersuchungsrichter als System der Kriminalistik* in which he advised law enforcement officers to preserve *corpus delicti* collected at the crime scenes in the air tight glass jars so as police dogs could sniff the scent of suspect from it and trail the perpetrator down. It was not certainly real scent identification line-up and the canines were used just to follow the track however it was a foundation on which the real scent identification by specially trained canines was later build in Europe, Czech lands included (Straus and Kloubek, 2008).

Probably the first case, where the police dog was used to pick a suspect in a line-up, was described to happen as early as in 1903. Canine officer Bussenius used his German shepherd Harras von der Polizei to identify a suspect in the murder case known later as the Duwe murder case (Schmidt, 1911). In 1904 the first German shepherd dogs, trained for police service, were imported by Russia. In 1907 the training center for police canine teams was founded and there are reports that the dogs were also used to identify detained suspects by using evidence collected at the crime scenes (Koschkin, 2007).

Similar practice was in effect also in the Czech territory. If a perpetrator threw away evidence on his escape from the crime scene it was often later used in the individual scent identification done by police dogs. In 1919 Friedo Schmidt (1910) published a book in which he described in detail how to collect evidence, bearing odor of the perpetrator, and store it in glass containers for the later identification by trained police teams. After long hesitation the Austrian Ministry of Defense approved in 1909 deployment of police dogs and in 1913 issued a directive regulating use of dogs. The directive contained guidelines how to collect odor traces at the crime scenes and how to use them in identification by dogs (Straus and Kloubek, 2008). The period from the Great War till 60s did not bring any further progress in terms of the individual scent identification (Eis, 1954; Krušinskij, 1954) nonetheless over the 60s the law enforcement agencies and forensic experts in Europe returned to the idea of storing odors, collected at the crime scenes, in glass jars and use them for the identification by specially trained dogs after some time when a suspect is detained.

In late 60s and 70s the method of the individual scent identification by specially trained canines was developing dynamically in the Soviet Union, Eastern Germany, Czechoslovakia, Poland and Hungary. It was already real scent identification method where the dogs were used to match odors collected from crime scenes with odors collected from the detained suspects. The results of the line-ups were not used in the courts of law as proof but were used exclusively by detectives to support other evidence in the search for perpetrators (Vyhnálek, 1985; Kloubek, 2007; Straus and Kloubek, 2008). Similar methods were in use

Fig. 1. Scent identification line-up in Eastern Germany 1973. (Courtesy of the Czech Rep. Police).

also in Western Europe e.g. Belgium, Netherlands, and West Germany (Kaldenbach, 1998; Schoon and Haak, 2002). Currently the results of the scent identification line-ups are accepted in courts of many European countries as circumstantial evidence the Czech Republic included (Teryngel, 2002; Schoon and Haak, 2002; Tomaszewski and Girdwoyn, 2006; Kloubek, 2007) .

In accordance with Straus and Kloubek (2008) who bibliographically reviewed and compared Czech and Russian scent identification line-ups by dogs it is probably Russia that has made the greatest progress in it compared to other European countries. In Russia the police canines are used and trained in close cooperation with scientific experts and innovative procedures like odor collection from blood samples are used. It seems that in sophisticated experiments 532 odor samples were tested by 12 trained dogs. Test included 16 years old samples of dried blood and even monozygotic twins (Filov, 2006; Koschkin, 2007). Unfortunately results of such researches and use in Russia are not published in English and they are not readily accessible even in original versions.

3.3. Discrimination of Identical Twins

As I have already mentioned, the experiments testing ability of dogs to distinguish between identical twins are not numerous and so I have decided to present them briefly in this theses.

Kalmus 1955

The first researcher who tested ability of dogs to discriminate scents of identical twins was British scientist Kalmus (1955) working for the Galton Laboratory of the University College in London. It was his reaction to Francis Galton's suggestion who as early as in 1875 recommended to test if dogs would be able to distinguish closely related twins by their scent. Kalmus had used nine dogs and in

his article he admitted that the dogs varied greatly in intelligence, perseverance and the degree to which they had been trained. Four of them were young male German shepherds trained for police duty by individual police officers. The other dogs were also police dogs a Doberman pincher, an old male Labrador and a female German pointer who was handled by more people. The last dogs were female German shepherds that were untrained but good at retrieving. The scent donors were seventeen men, nine women and five children.

The author did in fact three experiments that he called retrieving experiment, tracking experiment and twin experiment. In the retrieving experiment marked handkerchiefs, one of which was scented for a few minutes in a person's armpit, were laid out in a straight line in the absence of dog and handler. The dogs were then commanded to walk over the handkerchiefs and to seek and bring back the scented one among unscented ones. Next step consisted of commanding each dog to pick correct handkerchief among handkerchiefs that had been scented by other people. Some of the dogs could not perform well but those that could were used in the twin experiments. In tracking experiments two patterns were used. In the first one a track layer together with a number of other people went across a field and after 100 yards the track layer dropped a handkerchief, cap or similar object. Then all the people fanned out and hid behind the edge of the field. The dog was supposed to follow the track to the object and then to pick the right track. In the second tracking experiment the track layer went into the field and there he stuck a stick and dropped an object. Then he went and hid at the edge of the field. Then another person started from the same point, went to the stick and went on to the edge of the field returning repeatedly and passing the stick four times. The dog again was supposed to follow the correct track not switching to the distracting tracks.

Retrieving experiment as well as both tracking experiments were used in the twin testing. Four pairs of apparently identical twins were tested, three females and one male. Three year old female German shepherd was used in the retrieving task on a pair of fourteen years old identical sisters and eighteen month old male dog of the same breed was used in the tracking experiments with thirty three years old identical brothers living separately and identical twin sisters aged thirty two, who were unmarried, living in one household. During the retrieving task the canine in fact could not discriminate scents of both sisters and indiscriminately retrieved the handkerchief which she came first across.

The similar test was performed on two identical sisters aged twenty three also living together while the same dog was used. The tracking experiment showed also great similarity between scents of identical twins however suggested that dogs might be able to discriminate identical twins if they have an opportunity to check both scents at the same time. Kalmus came to a conclusion that in retrieving experiments dogs could not perceive any difference between identical

twins however they were able to do so if body odors of identical twins were offered to a well trained dog simultaneously.

Hepper 1988

Peter G. Hepper (1988) of the Queen's University of Belfast based his experiment on the findings of Kalmus that dogs are able to distinguish scents of identical twins if they are presented with both odors simultaneously. He used four dogs, two of them German shepherds, one Golden Labrador (probably Golden retriever), and a cross bred. As scent donors a set of identical as well as nonidentical twins was used. As a scent carrier washed, white T-shirts that had been worn by the twins for 24 hours were used. The dogs were to discriminate between two T-shirts placed in a plastic trough 45cm x 22,5cm x 22,5cm, after sniffing the T-shirts they were supposed to match. In accordance with the author: "To reduce the effect of environmental factors, all individuals used the same soap and T-shirts were washed in the same washing powder. After each T-shirt had been worn for 24 hours it was placed in a plastic bag and sealed until required. The T-shirt was only handled with plastic gloves after this". The author however did not give any other details on how the T-shirts were handled, how many persons assisted in it and if it was always the same person who put the T-shirts into the trough or if there were more of them. After evaluating the results Hepper concluded that: "Twins are discriminable by dogs if they differ genetically, or, if identical, they are subject to difference in their environment, particularly diet. However, if they are both genetically identical and fed the same diet then, to dogs at least, they do not produce sufficiently different odors to make them discriminable".

Sommerville, Green, and Gee 1991

Barbara Sommerville and her coworkers Michard Green and David Gee (1990) used in their experiment polyester squares worn by scent donors under the sleeves of T-shirts. Armpit sweat was later extracted from the patches. The special apparatus was then used to remove volatiles from the squares and concentrate them. As donors unrelated people as well as monozygotic twins were used. Chromatograms of the sweat samples were compared. Later the fraction of sweat, in which individual differences had been noted, was removed and used in testing with a dog. The chromatograms showed that there was a region showing certain individual variations in pattern. This pattern remained constant for several weeks and the variations in pattern were more similar in identical twins than in unrelated people.

The authors used only one dog, German shepherd, to match odors. The fabric squares caring tested odors were placed over the top of a disposable plastic cup containing warm water. The cup

was covered with an upturned plastic pot perforated by holes so that the evaporated water carried molecules of odors to the dog's nose. The dog was trained to sniff at the smeller sample and then to retrieve a matching sample. When the odors of unrelated people were used then three samples were presented to the dog in a line-up and two samples when matching odors of identical twins.

The dog did not performed very well in comparison to what the law enforcement canines are supposed to show nowadays. It made only 13 correct matches out of 17 for unrelated people. On the identical twins it matched correctly 14 times out of 21 that is a result slightly above the random score. When the dog was presented with the sweat fraction that seemed to display individual differences, it matched correctly samples of unrelated people in 11 cases out of 14. The samples of identical twins were retrieved indiscriminately.

Harvey et al. 2006

Lisa M. Harvey, Serina J. Harvey, Michele Hom, Avi Perna and John Salib (2006) from Victor Valley College in California used bloodhounds to determine what role plays genetics and what environment in the individual human scent. In the first test the bloodhounds were presented with a scent and then led by a handler to match the smeller with the trail on the ground. In the second test the bloodhounds were used to follow trails laid down by couples of persons. The trail split after some distance and dogs were supposed to follow the correct trail and then at the end alert to a correct person. As the trail layer couples of monozygotic twins, related persons as well as non related persons, living together and apart, were used. Specially designed vacuum scent collector had been utilized so as the scents of all individuals could be stored and later used as a smeller that was given to the bloodhounds to sniff before the trailing.

In the first test according to the authors: "Monozygotic twins appeared to be the most difficult group for the bloodhounds to differentiate correctly. There were no dogs that were able to perform better than chance when trailing the twins who lived together. When trailing twins who lived apart for a year or more, there was only one dog out of nine that performed better than chance. There was no significant difference between the performance of the bloodhounds trailing twins living together or apart". The dogs performed significantly better on all other groups of track layers whether living apart or together.

In the second test where two tracks were presented to the bloodhounds simultaneously, they performed better then in the first test. Three bloodhounds out of nine were able to perform better than chance when trailing monozygotic twins living together and five out of nine were able to perform better than chance when trailing monozygotic twins living apart. On all other groups the bloodhounds again performed significantly better.

3.4. Biology of Twinning

There exist two types of twins. Even people who are not familiar with twinning biology know very well that there are twins resembling each other as pods of peas and twins who look just like any other siblings. Identical appearance of identical twins has very often been used in novels, movies, and theater plays. As everyone knows twins might look so alike that they are actually indistinguishable by other people and sometime even by their own parents (Bulmer, 1970).

Identical or MZ (monozygotic) twins result when zygote divides during the first 2 weeks after conception. MZ twins are in fact genetic duplicates and have exactly the same genomes (Bryan, 1992). MZ twins represent currently in the Czech Republic 1/3 of all twin gestations. Depending on the age when the division occurs there are three types of physiologically developing twins:

- Dichorionic diamniotic – fetuses have their own placentas. At the 7 weeks of gestation two isolated chorionic cavities can be observed by US (ultra sound) screening.
- Monochorionic diamniotic – fetuses have only one placenta. Amniotic cavities are separated.
- Monochorionic monoamniotic – fetuses have only one placenta and amniotic cavity, however this type of MZ twins is very rare (Pašková, 2007).

Fraternal or DZ (dizygotic) twins are result of separate fertilization of two eggs by two spermatozoa (Bulmer, 1970). DZ twins thus have only 50% of genetic material in common just like any other siblings. DZ twins may be of the same sex or opposite sex as well. DZ twins represent currently in the Czech Republic 2/3 of all twin gestations. DZ twins are always dichorionic and diamniotic (Pašková, 2007).

Determination of twin type is done by the resemblance of the twins, US (ultrasound) screening, by comparative analyses of multiple

Fig. 2. Monozygotic twins. (Archive of the author).

blood group system analysis, and by DNA profile analyses. US screening can serve as a reliable diagnostic tool only if used within the first trimester of gestation. Blood group system analyses provide also reliable results especially when in combination with physical measures (Lykken, 1978). DNA profile analyses is however becoming increasingly popular as it is the most reliable of all above mentioned and it is also becoming less expensive (Richards et al., 1993).

4. Animals, Material, and Methods

4.1. Scent Identification in the Czech Republic Police

Scent identification in the Czech Republic is done by specially trained canine teams assigned to the canine units that are parts of the regional headquarters of the Czech Republic Police in the Capital city of Prague, Central Bohemian Region, Plzeň, Ústí nad Labem, České Budějovice, Hradec Králové, Ostrava, and Brno.

All handlers involved in the scent identification are sworn officers. To become a scent identification canine officer the applicant has to have at least 3 years practice as a regular patrolling canine handler. Then the chosen handlers go through the 3 month special course for the scent identification at the Canine Enforcement Training Center. Those that successfully graduate from the course are certified as scent identification canine specialists.

The scent identification is a method used in accordance with the section 1 of the Code of Criminal Procedures n. 141/1961 Sb. Scent traces are collected as evidence at the crime scenes by crime scene technicians. The crime scene technician puts specially designed sterile cotton squares (ARATEX™) that well absorb odors, on the objects and places at the crime scenes where scent of the perpetrator is supposed to be. The square is put on the

Fig. 3. Cotton square ARATEX™ used as a scent carrier by the Czech Republic Police for the scent identification procedures. (Pinc L).

object, covered by and aluminum tin foil and let for at least 30 minutes to absorb the odor. Scent traces can be collected from the ground, weapons, handles, etc, and even from the discharged casings, dead bodies, or objects recovered from water. Squares of ARATEX™ are put into a glass jars with twist off lids, labeled and sealed in special plastic bags with a bar code. Glass jars are stored at the canine units for later scent identification.

If a suspect is detained the scent is collected from him/her and also stored in the same glass jars that are also labeled and sealed in the plastic bags. The suspects are to wash their hands and put the

squares of ARATEX™ on their body by themselves or this is done by a crime scene technician using sterile tongs. If a suspect refuses scent collection his/her resistance can be overcome by force. The persons present at the crime scene cannot assist in scent collection from the suspect.

The Bureau of Criminal Investigation then sends the glass jars collected from the suspects to the canine unit and asks for the scent identification line up to match the odors collected at the crime scene with the odor collected from the suspect. The SIC officers are usually provided with the basic information about the case. The SIC officer is then supposed to organize and perform the line up by himself/herself and write down an official report in which he/she describes how the line up was performed and with what result. The target odor is placed in a line of seven glass jars in total. The handler gives one odor to the dog as a smeller and then sends it or leads it along the line. The dog either alerts to the target scent or passes it without an alert. In accordance with the regulations the dog is on leash, off leash or the handler can use top line connected with a running leash. Prior to the intrinsic identification the target scent is placed in the line as an attractor. The canine officer uses previously collected scent as another smeller and another target scent that is placed behind the attractor. If the dog alerts to the attractor it means that there is something in the scent that attracts the dog's attention and the identification is canceled. If the dog does not pay any attention to the attractor the identification goes on. The other glass jars in the line contains odors called distractors. As the distractors the scents of the same sex and similar age, race and backgrounds are used. The distractors should be also of the similar scent intensity as the target odors. As I have already mentioned the organization of the identification procedure is upon the canine officer. It also means that the officer decides which scent is used as a smeller (starting odor) and which is used as the target scent (placed in the line). In the scent identification line ups also two scents from the crime scenes can be matched as well as two scents collected from persons.

Fig. 4. ARATEX™ squares are always handled in sterile tongs. (Pinc L.)

The result is always positive or negative i.e. the canine matched the odors or did not match the odors. To pronounce the result positive the dog has to match the odors positively three times, for the negative result the dog has to pass the glass jar in a line up without an alert twice. The result of such identification line ups are widely accepted by the Czech Courts of Law as circumstantial

evidence. It means that the scent identification itself is not sufficient to prove the suspect guilty however there has to be other supporting evidence. Scent collection as well as scent identification is done in accordance with the Police President's Direction Num. 52 of April 25, 2007 which constitutes unified principles of the scent identification use in the Czech Republic Police (Závazný pokyn policejního prezidenta č. 52, kterým se stanoví zásady k zabezpečení jednotného postupu Policie České republiky při využívání metody pachové identifikace.).

4.2. Scent Identification Canine (SIC) Training and Certification

To become a SIC handler the applicant has to be a sworn police officer with at least three years experience in law enforcement canine handling and training. The breed and sex of SIC are not strictly given however most of the canines used by the Czech Republic Police are female German Shepherds. It does not mean that other breeds would not be suitable but the current breed and sex distribution of SICs reflects the fact that the Czech Republic Police have their own breeding facilities producing solely German Shepherds. Males are most frequently deployed as patrolling or tracking canines because of the body size and more self-confident temperament and so females are frequently used as detector or scent identification canines. Canines for the scent identification are carefully selected as most of the training takes place at the scent identification bay in more or less close quarters which asks for the dogs of sound

Fig. 5. The scent identification canine team on the job. (Pinc. L)

and well balanced temperament. To eliminate the stress resulting from the work in the scent identification bay the dogs are frequently exercised outdoors, run next to a bicycle etc.

The scent identification bays are rooms of rectangular shape with or without windows illuminated electrically, ventilated or equipped with air conditioning. Microorganisms are inactivated by regular disinfection and germicide radiators that are switched on when the scent identification is over. In the bays there are usually mounted camcorders to record line-ups. The glass jars are stored in special store rooms under normal room temperature. Scent identification

facilities are also equipped with apparatuses and appliances to wash and sterilize glass jars, lids, and tongs and sterilize cotton squares for the scent collection.

Fig.6 . Scent identification bay at the Czech Republic Police canine unit. The bays are equipped with air conditioning, ventilation and radiators for the germ inactivation. (Pinc L)

To become certified the novice canine teams (handlers with their canines) have to go through 15 weeks elementary course at the canine academy and at the end of the course successfully accomplish three training cases. The handlers are allowed to arrange the glass jars with the scents by themselves, however they do not know if the results of the line-ups are supposed to be positive or negative. After finishing the line-ups they write and sing reports with the final results. To become certified the canine has to perform absolutely flawlessly and no error is allowed. After the certification the canine teams serve at the canine units under regional police HQ. Once a year all SIC teams return for the 3 weeks recertification course to the canine academy were the dogs are recertified. At the end of the course the handler with the dog has to successfully carry out two training cases and again no mistake is allowed. New dogs with certified handlers have to go through the 10 weeks certification course.

The training in scent identification usually starts with the dogs that are older than 18months. The basic training can be divided into several steps:

1) **Sniffing at the glass jars in a scent identification bay.**

At the very beginning the dog is trained to sniff at the glass jars in the scent identification bay. The handler drops a tidbit into each of seven glass jars in a line and at the same time he/she has additional 7 tidbits ready in his/her hand. The dog is led along the line of the glass jars and after sniffing at each glass jar the handler drops a tidbit from the jar for it. Then he/she leads the dog to another jar and at the same time puts a new tidbit into the one that has been emptied. The manipulation with the tidbits has to be done as fast as possible so as the dog would not pay any attention to it. At the end of the line the handler with the dog turns around and goes back doing the same manipulations with the tidbits. On the way back the new tidbits are not dropped into the jars. As soon as the dog starts sniffing actively at the jars, the handler gives command to down at each jar with the tidbit. Immediately after the dog lies down it is rewarded by dropping a tidbit from the glass jar. When the dog starts responding quickly and actively to the tidbit in a glass jar by lying down, the tidbits are placed only into 3 – 4 glass jars. Similarly the dog is trained to sniff at the scent carrier (ARATEX™) held in the tongs. At the beginning the dog is trained to sniff at the square of ARATEX™ held in a bare hand in which there is also a tidbit.

2) **Handler's scent among odorless scent carriers.**

Now when the dog sniffs actively at the cotton squares held in the tongs as well at the glass jars, it is time to train it to match odors. The handler uses two cotton squares caring his/her own scent. One is used as a smeller (starting odor held in tongs) and the other is placed as a target scent in the line of glass jar with odorless squares. The handler's scent is connected with a tidbit. When the dog responses to the glass jar with the target scent, by lying down, handler drops the tidbit from the glass jar for it. Later the tidbit is not in the glass jar anymore and handler rewards the dog with tidbit from hand. The position of the target scent in the line is always changed.

3) **Handler's scent among strange ones.**

As soon as the dog manages to match handler's own cent correctly it is time to put distracting odors into the glass jars. At the beginning it will be only one unknown odor and later there will be different distracting odor in each glass jar. To make it easier for the dog the target scent will be strong while the distracting odors will be weak. Later the smellers and target scents will be weaker and weaker and the distractors stronger and stronger. Time to time there will be no positive match line however the session will always ends with positive alert.

4) **Strange scent among strange ones.**

The training goes on similarly as in previous step. The starting scent as well as the target one is strong while the distraction scents are weak. Later the intensity of scents is changed. The scent collected not only from persons but also from various surfaces and objects are used. As starting scents the scents collected from surfaces can be used and matched with other scents collected from surfaces both of various intensity.

5) **Suppressing dog's memory.**

Finally the dog is trained to response only to the scent used as a smeller in the actual line-up. It means that the target scent that was used in the last line-up is left in the line of glass jars however the dog is not allowed to respond to it again but only to the actually used starting scent.

6) **Unbiased approach.**

The necessity to avoid influencing the dog during the scent identification is emphasized in the police regulations however the dogs can take as clues the faintest behavioral changes that are handlers not even aware of (Schoon and Haak, 2002). It means that there is a necessity to change the behavior deliberately during the line-ups so as the dog would not take the handler's behavior changes as possible clues for the correct alert. It is also necessary so as the handlers assigned to the scent identification teams cooperate closely in the training, especially in scent samples collection and line-ups arrangements so as they often would not know about the correct result in advance.

7) **Physical condition and relaxation.**

To compensate for the excessive stress resulting from the monotonous scent identification in the close quarters of the training bay the canine officers take their canines often outdoors for walks and relaxing physical exercises.

4.3. Canines and Material Used in the Research

4.3.1. Twins

Totally I have collected scents from 16 couples of identical as well is nonidentical twins, however in the experiment I used only two couples of identical (monozygotic) (n=2) and two couples of nonidentical (dizygotic) (n=2) twins. I have used the youngest couples I managed to get a hold of with the highest probability of the correct zygosity diagnosis.

Monozygotic twins

1) The couple labeled as number 5. They were two males at the age of 5 years. Monozygosity was diagnosed by ultrasound screening in the first trimester of gestation. The boys look alike and share the same blood groups as well as the first 5 blood subgroups. Both live in the same household and eat the same food. Both children underwent varicella and twin "A" had been treated for the adenoids inflammation.

2) The couple labeled as number 13. They were two females at the age of 7 years. Their mother who is a gynecologist diagnosed the monozygosity with ultrasound screening by herself in the first trimester as early as in the 4-5 week of gestation. In accordance with her statement the children were monochorionic, diamniotic. The children look alike. They eat exactly the same food and live certainly in the same environment. The twin labeled "A" had been treated for Lyme disease.

Dizygotic twins

1) The couple labeled as number 6. The children are males 13 years old. They were diagnosed as dizygotic in the first trimester of gestation. The children look different. They live in the same household. The twin labeled as "B" does not eat vegetables.

2) The couple labeled as number 8. The children are females at the age of 8 years. They were diagnosed as dizygotic by their mother who is a doctor in the first trimester of gestation. The children look different and have different food preferences. The twin "A" prefers meat while the twin "B" prefers cereals and yogurts. They live in the same household.

4.3.2. Canines

All dogs (n=10) used in the experiment were scent identification police canines owned by the Czech Republic Police that serve with their handlers at the three different regional police headquarters – in Brno, Hradec Králové, and Plzeň. All canines were pure bread German Shepherds. Most of the dogs were females (n=7), the rest (n=3) were males. The canines routinely perform scent identification line –ups as a part of criminal investigation procedures. The only exception was young uncertified canine RONY that was in the middle of the scent identification elementary course in the Canine Enforcement Training Center in Plzeň Bílá Hora. This dog, however, matched scents of only one

couple of identical twins and did not participate on the experiment. I included his line-up result in my study as it was the only canine that matched positively scents of identical twins couple. The names of the participating canines are listed below:

Regional Czech Republic Police HQ Brno

GABI (female)

KORA (female)

Both of these canines were handled by the same canine officer

Regional Czech Republic Police HQ Hradec Králové

YVERA (female)

MIRA (female)

Both of these canines were handled by the same canine officer.

YARA (female)

UMA (female)

Both of these canines were handled by the same canine officer.

Regional Czech Republic Police HQ Plzeň

NUK (male)

EVAN (male)

Both of these canines were handled by the same canine officer.

NADIR (male)

JEFFRA (female)

Both of these canines were handled by the same canine officer.

4.4. Methodology

4.4.1. Scent Collection

All scent samples were collected and stored according with the protocol routinely used by the crime scene technicians when collecting scent samples from suspects. All experimental subjects were children old enough so as they could open glass jars and apply the ARATEX™ cotton squares by themselves. Before the scent collection the twins were separated into different rooms and then given the glass jars containing ARATEX™ squares that they put by themselves on the naked skin in the

belly region. Then they put clothing back over the squares and let the squares absorb their scent for 20 minutes. In the scent collection always assisted two different people so as to avoid cross contamination and twins were not allowed to get in touch. During the scent absorption the children were asked to put the lid back on the glass jar. After 20 minutes they put the square with the scent back into the glass jar and the adult assistant tightened the lid. Then the glass jar was labeled. Two scent samples were collected from each twin. Then the glass jars were transported to an office at the Czech University of Life Sciences where they were stored in room temperature i.e. in the similar conditions as the scent samples are stored at the police canine facilities. Similarly to the twin scent collection, distracting scent samples were collected from the children in the Elementary School in Újezd nad Lesy, however the children were not separated into different rooms and were only prevented from getting into contact with each other. Distracting scent samples were collected from 5 boys at the age of 6 to 7 years and from 5 girls also at the age of 6 to 7 years.

All glass jars and ARATEX™ squares, used in the experiment, were from the police supplies ready for the use in criminal identification procedures and so the glass jars as well as the squares were sterilized at the police scent identification canine facility. Glass jars were labeled with the labels used in the chain of evidence protocol. The scent samples were listed by the numbers of scent collection (twin couple number) i.e. 5 and 13 for identical and 6 and 8 for nonidentical twins and as "A" for one twin and "B" for the other in the couple.

4.4.2. Line-ups

The intrinsic scent identification took place at the police scent identification canine facilities that are parts of the police canine HQ in Brno, Hradec Králové, and Plzeň, and also in the Canine Enforcement Training Center in Bílá Hora in Plzeň. Some of the line-ups were supervised by the author but in most cases by the chief of the above mentioned canine academy.

Handlers were not aware of the experiment details and did not know anything about expected results. They were given the glass jars with the scent samples and asked to do the scent identification and write down an official report on the outcome of the scent identification line-ups just like in the case of regular criminal investigation. The handlers were in some cases allowed to arrange the glass jars but in most cases this was done by the chief of the canine academy. The scent samples were used repeatedly in accordance with the regular practice however in the past the scent samples were never used so many times. Prior to the line ups the lids of the glass jars had been removed and then the glass jars containing the ARATEX™ squares were arranged in the lines among the glass jars with the distracting scents.
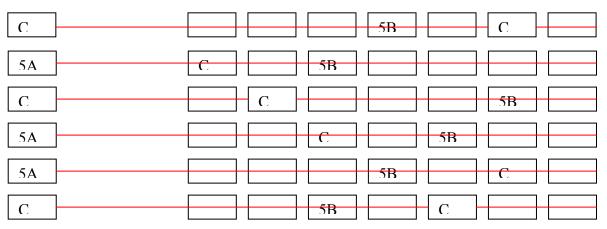
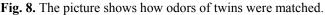All handlers used also training cases scents as controls in the line-ups. The training cases scent had been collected by the handlers themselves for the training purposes. In the matching procedures each handler placed one twin scent into the line of distractors as so called attracting scent. Then he/she placed a training scent behind the atractor. In the first line-up the dog had to pass the twin odor (atractor) without alerting to it. This is a proof that the odor itself is not attracting to the dog. Then the handler opened the glass

Fig. 7. The scent identification canine signals positive match by lying down. (Pinc L)

jar containing an odor of the other twin and used it as a smeller (starting scent). The control scent previously used was usually left in the line. Next line-up the dog again matched control scents while the twin scent was left in the line however the position of twin scent as well as of the control scent was changed after the each line-up. Then the procedure was repeated. The matching procedure of the two twin odors usually ended by matching two control scents. The same protocol was used when the dogs matched two odors collected from the same twin. For the better comprehension the scent identification diagrams are showed bellow.

Fig. 8. The picture shows how odors of twins were matched.

The red line shows movement and alerts of the dog.

"C" is a control scent or training scent used by the handler for the training purposes to let the dog make positive match.
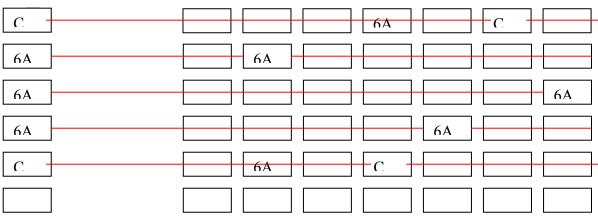
"5A" is a twin scent used as a smeller (starting odor).

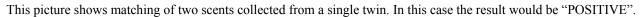"5B" is a twin scent used as a target scent.

The blank rectangles stand for the distracting odors. Interrupted red line means that the dog alerted to the odor. Note the "5B" sample in the first line used as an atractor.

A result of this line-up would be NEGATIVE i.e. the dog did not match positively the two odors.

All handlers did not follow exactly the same pattern and for some of them two line-ups without the dog's alert were enough to state that the result was "negative".

Fig. 9.
This picture shows matching of two scents collected from a single twin. In this case the result would be "POSITIVE".

As there are more than 100 diagrams showing performances of dogs in this experiment, which would take 40 pages, they were not attached to this thesis and are stored with the author. The results of the line-ups are in shortened form showed in the tables below (Chapter 5, Results).

5. Results

Performance of the dogs is depicted in the tables below. Each table consists of the line-ups of one twin couple done by one dog. The smeller means a starting odor. The target is an odor placed in the line of distracting odors. A result of the scent identification is indicated by "X". Unfilled lines in the tables mean that the dog did not do the line-up. Each result means that in accordance with the Czech Police regulations for the positive match require the dog to work out at least three-line ups with positive alert and for the negative match the dog has to pass at least twice the line of glass jars without alerting to the target odor.

To evaluate the results statistically the Sign test was used. The Sign test is a special case of the binominal test where the theory is that the two outcomes have equal probabilities. The binominal test is used when there are two possible outcomes (GraphPad, 2008). The two probable outcomes are showed in the tables bellow as "correct" and "incorrect". To compute the results GraphPad Software by GraphPad Software Inc. was used (http://www.graphpad.com/quickcalcs).

All dogs flawlessly discriminated scents of monozygotic as well as dizygotic twins. Two scents collected from single twins were also in all cases correctly matched.

Thus the results did not show any variation (The Sign test, P<0.001).

Tables with the scent identification outcomes

TABLE 1

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
YVERA	5A	5B		X	X	
	5A	5A	X		X	
	5B	5B	X		X	

TABLE 2

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
YVERA	13A	13B		X	X	
	13A	13A	X		X	
	13B	13B	X		X	

TABLE 3

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
YVERA	6A	6B		X	X	
	6A	6A	X		X	
	6B	6B	X		X	

TABLE 4

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
YVERA	8A	8B		X	X	
	8A	8A	X		X	
	8B	8B	X		X	

TABLE 5

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
MIRA	5A	5B		X	X	
	5A	5A	X		X	
	5B	5B	X		X	

TABLE 6

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
MIRA	13A	13B		X	X	
	13A	13A	X		X	
	13B	13B	X		X	

TABLE 7

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
MIRA	6A	6B		X	X	
	6A	6A	X		X	
	6B	6B	X		X	

TABLE 8

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
MIRA	8A	8B		X	X	
	8A	8A	X		X	
	8B	8B	X		X	

TABLE 9

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
YARA	5A	5B		X	X	
	5A	5A	X		X	
	5B	5B	X		X	

TABLE 10

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
YARA	13A	13B		X	X	
	13A	13A	X		X	
	13B	13B	X		X	

TABLE 11

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
YARA	6A	6B		X	X	
	6A	6A	X		X	
	6B	6B	X		X	

TABLE 12

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
YARA	8A	8B		X	X	
	8A	8A	X		X	
	8B	8B	X		X	

TABLE 13

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
UMA	5A	5B		X	X	
	5A	5A	X		X	
	5B	5B	X		X	

TABLE 14

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
UMA	13A	13B		X	X	
	13A	13A	X		X	
	13B	13B	X		X	

TABLE 15

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
UMA	6A	6B		X	X	
	6A	6A	X		X	
	6B	6B	X		X	

TABLE 16

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
UMA	8A	8B		X	X	
	8A	8A	X		X	
	8B	8B	X		X	

TABLE 17

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
NUK	5A	5B		X	X	
	5A	5A	X		X	
	5B	5B	X		X	

TABLE 18

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
NUK	13A	13B		X	X	
	13A	13A	X		X	
	13B	13B	X		X	

TABLE 19

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
NUK	6A	6B		X	X	
	6A	6A	X		X	
	6B	6B	X		X	

TABLE 20

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
NUK	8A	8B		X	X	
	8A	8B	X		X	
	8B	8B	X		X	

TABLE 21

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
JEFFRA	5A	5B		X	X	
	5A	5A	X		X	
	5B	5B	X		X	

TABLE 22

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
JEFFRA	13A	13B		X	X	
	13A	13A	X		X	
	13B	13B	X		X	

TABLE 23

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
JEFFRA	6A	6B		X	X	
	6A	6A	X		X	
	6B	6B	X		X	

TABLE 24

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
JEFFRA	8A	8B		X	X	
	8B	8B	X		X	

TABLE 25

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
NADIR	5A	5B		X	X	
	5A	5A	X		X	
	5B	5B	X		X	

TABLE 26

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
NADIR	13A	13B		X	X	
	13A	13A	X		X	
	13B	13B	X		X	

TABLE 27

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
NADIR	6A	6B		X	X	
	6A	6A	X		X	
	6B	6B	X		X	

TABLE 28

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
NADIR	8A	8B		X	X	
	8A	8A	X		X	
	8B	8B	X		X	

TABLE 29

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
EVAN	5A	5B		X	X	
	5A	5A	X		X	
	5B	5B	X		X	

TABLE 30

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
EVAN	13A	13B		X	X	
	13A	13A	X		X	
	13B	13B	X		X	

TABLE 31

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
EVAN	6A	6B		X	X	
	6A	6A	X		X	
	6B	6B	X		X	

TABLE 32

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
EVAN	8A	8B		X	X	
	8A	8A	X		X	
	8B	8B	X		X	

TABLE 33

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
GABI	5A	5B		X	X	
	5A	5A	X		X	
	5B	5B	X		X	

TABLE 34

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
GABI	13A	13B		X	X	
	13A	13A	X		X	
	13B	13B	X		X	

TABLE 35

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
GABI	6A	6B		X	X	
	6A	6A	X		X	
	6B	6B	X		X	

TABLE 36

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
GABI	8A	8B		X	X	
	8A	8A	X		X	
	8B	8B	X		X	

TABLE 37

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
KORA	5A	5B		X	X	
	5A	5A	X		X	

TABLE 38

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
KORA	13A	13B		X	X	
	13A	13A	X		X	

TABLE 39

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
KORA	6A	6B		X	X	

TABLE 40

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
KORA	8A	8B		X	X	

TABLE 41

Canine	Smeller	Target	Positive	Negative	Correct	Incorrect
RONY	5A	5B	X			X

RONY was the only canine used in the experiment that had not been certified and in the time of the line-up was in the middle of the elementary scent identification course in the canine training center. Canine KORA did not finish the experiment for the medical reasons however she successfully accomplished matching the scents of identical as well as nonidentical twins. She did not finish matching two odors collected from the individual twins.

6. Discussion

The findings of this study are in contradiction with my hypothesis, based on the earlier studies suggestions, that dogs cannot discriminate individual scents of human identical twins living in the same environment unless both scents are presented to them simultaneously (Kalmus, 1955; Hepper, 1988; Harvey et al., 2006). In the previous studies the authors accordingly concluded that people have individual odorotypes as a result of their genomes. Numerous studies showed that MHC plays a decisive role in olfactory individual recognition olfactory kin recognition as well as in reproductive behaviors in mice and other animals (Ferstl et al., 1998; Eggert et al., 1998; Thom et al., 2005). Apparently MHC may play a role even in human scent attractiveness (Thornhill et al., 2003; Santos et al., 2005). As monozygotic twins are supposed to have identical MHC genes it seemed not to be surprising that dogs were not able reliably distinguish one identical twin from the other. The previous studies also showed that at least some dogs were able to differentiate monozygotic twins provided that both odors were very close to each other (Kalmus, 1955; Harvey et al., 2006). It shows that there might be individual odorotypes, as a result of very polymorphic MHC genes, however in case dogs simply have to decide which odor is the better match, they are able to do so nevertheless under such circumstances they use secondary or tertiary odors (Curran et al., 2007) as auxiliary clues. This explains why the Czech police canines were able to discriminate scents of identical twins.

Unlike other studies that I have cited in this thesis, dogs used in my research were police dogs trained, certified and routinely used exclusively for scent identification line-ups. Some dogs used by Kalmus (1955) were also trained police canines however not exclusively for scent identification, moreover the level of efficacy was not uniform and author used only those that performed best. Yet we cannot say that that the dogs were certified and trained SICs.

Hepper (1988) described in details how the scents of the persons were collected and how they washed and avoided using perfumes and deodorants etc. but he did not offer any information on the four dogs that were used in the study safe for their names, breeds and ages. He did not describe how the dogs were trained and if they had any previous experiences with scent identification.

Sommerville et al. (1990) used in their study only one dog and they did not describe the way how the dog was trained either however the dog did not perform entirely flawlessly.

Bloodhounds used by Harwey and coworkers (2006) were also police dogs nevertheless they were not trained and certified to perform line-ups either. The fact that dogs were able distinguish two trails of identical twins laid down one next to the other suggests that dogs do have potential to

distinguish individual scents of MZ twins and the fact that they had problems to match scent presented to them by a handler with the correct single trail on the ground means that the scents of MZ twins are similar but not the same. The only example of partly trained dog that matched positively scents of MZ twins only supports this assumption. However it is true that single dog with single line-up cannot be used as a proof but only as an indication that yet has to be supported by further investigations.

Ability of the Czech police dogs to match crime scene scents stored for several years in glass jars with scents, collected from detained suspects several years later, suggests that there is however a human odor signature that remains unchanged for a long time as the suspects may change their food preferences, start or quit smoking etc. and yet the dogs are able correctly match their scents (Kloubek, 2007; Bukvaj, 2007). Czech police SICs undergo very intensive training focused on their ability to reliably discriminate odors. Talented and experience handlers are trained in the training facilities that have more than thirty years lasting tradition in it. All above mentioned details show that the results achieved by Kalmus (1955), Hepper (1988), Sommerville with coworkers, (1990), and by Harvey et al. (2006) are contradictory with my results because of the fact that the efficiency and approach to the training of the dogs used in my study are different.

Last but not least the study shoved that if the Czech Republic Police Scent Identification Protocol is observed, the glass jars with the scents can be used repeatedly which is in contradiction with the warning of Schoon and Haak (2002) that if the scent carrier is used repeatedly the moisture in the dog's breath will ruin the scent and it cannot be used anymore. In this study the actual numbers when the scent samples were used has not been recorded and in "A" – "B" line ups the scent carriers were used randomly, nevertheless it was undoubtedly almost 70 times in total (not to mention the cases when the jars were left in lines as distractors or atractors) as each dog did at least 2 line –ups for the negative and 3 for the positive result. The truth is that the Czech police dogs usually do not stick their noses into the glass jars. The smeller is by most handlers held in the sterile tongs and the dog sniffs at it without touching it. While walking along the line of glass jars the well trained dogs do not stick their noses into the jars and it often looks like the dog does not actually sniff till it alerts. As the dogs involved in the experiment were engaged in the real criminal investigations it was not possible to finish the experiment within a short time and so the glass jars containing scent carriers (ARATEX™) had been used for 7 months. Yet the dogs were able to successfully finish the line-ups with the same scent samples.

7. Conclusions

The results of this study show that in spite of the fact that identical twins posses identical MHCs there are either endogenous or exogenous processes that change individual odor signature of each individual over the early ontogeny and thus enable dogs to discriminate one identical twin from the other even if they live in the same environment. In case of criminal investigation where identical twins are involved the reasoning that law enforcement canines are not able to distinguish individual scents of them cannot be therefore accepted. It has been shown that even school children living in the same household can be distinguished however by properly trained canines. Earlier findings together with an example of one partly trained dog, mentioned in this study, show that nonetheless identical twins have individual odors that are much more similar then individual odors of fraternal twins as well as other related persons to say nothing of the unrelated individuals. This study also shows that if the Czech Republic Police scent identification protocol is followed the scent carriers can be used repeatedly without deteriorating the scent qualities.

Scent identification canine seems to be very efficient and powerful tool to combat crime. All law enforcement personnel nevertheless has to bear in mind that even the best trained police canine is still only an animal and it is always a human that is supposed to evaluate and correctly interpret the results achieved with the help of the for-legged partner. Currently there is no way how the performance of the dog could be controlled as there is no technology that would enable police officers to collect and identify odors from the crime scenes. The only way how to control a dog is another dog. Despite of all the progress that has been achieved in recent decades we still do not know for sure which structures and mechanisms are responsible for individual scent in humans and if there is really an individual human odor signature that would not change through the lifetime regardless of all endogenous or exogenous factors. It remains also unknown whether individual scents of identical twins differ as early as immediately after parturition and if so, when they start to differ enough so as they could be discriminated by dogs.

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