Journal of Veterinary Behavior (2011) 6, 150-157

ELSEVIER

Journal of
Veterinary Behavior
Clinical Applications
and Research

RESEARCH

Evaluating the effect of early neurological stimulation on the development and training of mine detection dogs

Adee Schoon[a], **Terje Groth Berntsen**[b]

[a]*ADConsultancy/Leiden University, Leiden, The Netherlands; and*
[b]*Global Training Centre, Norwegian People's Aid, Vogošća, Bosnia and Herzegovina.*

KEYWORDS:
early neurological stimulation;
socialization;
puppies;
test;
working dogs;
training

Abstract Early neurological stimulation (ENS) has been proposed to enhance the natural abilities of dogs. This kind of stimulation involves subjecting pups aged between 3 and 16 days to mild forms of stimulation leading to "stress," and is said to lead to faster maturation and better problem-solving abilities later in life. ENS resulted from a U.S. Military program called Bio Sensor, and is currently being used in some other working dog programs. It has been part of the breeding program for mine detection dogs at the Global Training Centre (GTC, part of Norwegian People's Aid) for 4 years.

To investigate the effects of ENS on the basis of a previous study (Battaglia, 2009, J. Vet. Behav.: Clin. Appl. Res. 4, 203-210), 10 litters born since the spring of 2008 at the GTC were randomly divided into the following 2 groups: (1) those receiving ENS, and (2) those receiving the same amount of human attention without being subjected to the ENS exercises. Developmental parameters were monitored by the kennel staff. The pups were subjected to testing at approximately 10 weeks of age by investigators who were blinded to treatment. Their careers as working dogs were monitored.

There was no observed effect of ENS on either the development of the pups when compared with those who were exposed to the standard GTC stimulation program within the same age range or on the later training results of the dogs in their careers as mine detection dogs. This lack of effect could well be the result of the very rich standards of the GTC socialization program that is given to these dogs.

Introduction

In both popular and scientific literature, the necessity of adequate socialization for the development of puppies into "successful" adults has been well described. Many of these studies can be traced back to the fundamental study conducted by Scott and Fuller in 1965, which stressed on the importance of the socialization period. This period starts at around 3 weeks of age, and is usually thought to last until around 12 weeks of age. It is clear that this period is important for developing behaviors that are essential for developing social relationships during the later life of the dog.

In a study on different socialization programs, Seksel et al. (1999) found no significant differences between programs that focused on both training and socialization, or on only training, or only socialization, even after comparing

Address for reprint requests and correspondence: Adee Schoon, PhD, Leiden University, PO box 9516, 2300 RA Leiden, The Netherlands; Tel: +31 578631683.
E-mail: animaldetectionconsultancy@gmail.com

1558-7878/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.jveb.2010.09.017

with a control group of dogs who were not given any specific program. However, these programs were very short (1 hour weekly for 4 weeks), the pups were started on these at the age of between 6 and 16 weeks, and the owners were not restricted in their further activities with the dogs. In fact, the first test that was conducted with the pups before enrolling them into the selected program seemed to be the best predictor of later behavior.

Studies suggest that the earliest period in the life of a developing animal seems to have more pronounced effects. In a study by Fox and Stelzner (1966), a comparison was made between a group of puppies raised in isolation after weaning at 4 weeks, a group that was provided an extremely large amount of handling from birth until about 5 weeks of age, and a control group that was given a "normal" amount of contact throughout (twice daily for a few minutes). Significant differences were found during testing at 5 weeks. The authors reported that in comparison with the other 2 groups, the puppies who were handled had a more mature electroencephalogram, they were better at problem solving tasks, were less "emotionally aroused" (described as a combination of distress vocalizations, random activity, and nonspecific exploratory [i.e., not focused on the toys in the arena]) in an arena test, were more "dominant" in a group (details not provided), and more attracted to people.

Other studies conducted with kittens and rabbits also demonstrated a marked effect of early stimulation on the development of their young ones. In a recent review, Battaglia (2009) described several such studies and concluded that early stimulation introduces animals to mild stress which stimulates the hormonal, adrenal, and pituitary systems, resulting in their being able to handle stress as adults in a more "graded" manner. In earlier articles, which are available on the Internet, he had stressed on the use of a particular set of exercises to provide early neurological stimulation (ENS) for the dogs. These exercises were adapted from the work conducted by the U.S. Military on their "Biosensor" program that aimed at raising excellent detector dogs in the 1970s. The assumption is that these exercises lay the foundation for a better neurological development of the puppies, which enables them to perform better as working dogs later in life.

Despite this logic, there are no published studies that have tested whether this particular set of exercises actually works. Stimulation to promote socialization among puppies has been incorporated into many breeding programs for dogs that are being raised to be working dogs. The question to be asked is whether ENS exercises enhance routine exposure and handling of dogs. Does it affect the development of puppies raised in a rich environment in any way, or benefit their learning abilities? This study sought to answer the question by using dogs that were bred and raised at the Global Training Centre (GTC) for mine detection dogs (MDD) part of the Norwegian People's Aid.

Materials and methods

Puppies

Since 2004, GTC has been conducting an ongoing breeding program for dogs of the breed Belgian malinois. A total of 81 puppies from 10 litters were used for this study. These puppies were born in 2008 between the months of February and July. Their parents were of the pedigree Belgian malinois, and a vast majority were trained MDDs (or had the potential for being one).

A stratified random design was used, where stratification was by sex. On the day after birth, for each litter, pups of each sex were randomly assigned to either the treatment group, where pups were subjected to daily ENS exercises, or to the control group, where pups were not subjected to the ENS exercises but were instead held in the arms of one of the kennel staff for the duration of the ENS exercises. This design controlled for the effect of human contact. In general, every pup in the litter was treated in the same way and exposed to the standard enrichment and socialization program at the GTC. An overview of the puppies and the experimental conditions that they were subjected to are provided in Table 1. A description of the ENS exercises and the socialization program is provided in the following paragraphs.

ENS exercises

For each puppy, a series of 5 exercises were executed one after another in a nonspecific order. From the past few years, details of these exercises have been available on the Internet, and have been recently published by Battaglia (2009).

1. Tactile stimulation: Using a Q-tip, the puppy activator tickles the puppy between the toes for a few seconds.
2. Head held up: Using both hands, the pup is held upright so that the head is directly above the tail for a few seconds.
3. Head pointed down: Using both hands, the pup is held upside down so that the head points toward the ground and the tail is above the head for a few seconds.
4. Supine position: Using both hands, the pup is held so that it is lying on its back, with its muzzle facing the ceiling for a few seconds.
5. Thermal stimulation: The pup is placed with all its limbs on a damp towel that has been kept in the freezer overnight for a few seconds and the pup is not restrained from moving.

Brief description of standard socialization program at GTC and the experimental conditions

Week 1-2

The puppies are placed in a litter box with their mother in a room. The radio is kept constantly on, and the minimum

Table 1 Allocation of puppies from 10 litters to the experimental (ENS) and control groups

		Number of females		Number of males	
Litter	Date of birth	ENS exercises	Control	ENS exercises	Control
W	February 4, 2008	4	4	2	2
X	February 14, 2008	1	1	1	1
Z	March 30, 2008	2	2	3	3
B	May 18, 2008	2	1	2	2
C	May 23, 2008	1	1	2	2
D	June 16, 2008	3	2	3	3
E	June 30, 2008	1	1	2	2
F	July 5, 2008	1	1	3	3
G	July 23, 2008	0	0	4	3
H	July 29, 2008	2	1	3	4
Total		17	14	25	25

temperature range is maintained at 12 °C-14 °C. Puppy "activators" enter the room several times a day, and the mother is taken away for a walk for a minimum of 3 times daily. The ENS exercises were conducted in the absence of the mother. At the time of subjecting a pup from the test group to the ENS exercises, a pup from the control group was held close to the body by another puppy activator for the duration of the exercises.

Week 3

Activity is increased. Puppy activators enter the room and talk to the puppies in a "happy voice", pick them up, and hold them close. When the weather is good, the door is left open so that the pups can hear more and people can look in the room. The first soft toys are introduced and the pups are given their first worm treatment at end of the week. All handling of pups was done in the absence of the mother. Each puppy from the ENS group was subjected to the exercises, whereas a control puppy was held by another puppy activator at the same time.

Week 4

The puppies are still kept in the litter box in the breeding room, but they can get out of it. The radio is still kept on constantly and the minimum temperature continues to be in the 12 °C-14 °C range. The mother is only present during the night and is brought in for feeding. Semisolid food is introduced, the puppies are made to eat together as a group, and the puppy activators touch the pups during feeding. The pups are introduced to more people and children during a minimum of 2-3 visits per day. Visitors feed the puppies small morsels of food, and also touch them. More toys are given to the puppies and the visitors play with them. The toys are not kept in the presence of the mother so as to prevent accidents in cases when the mother may want the toys for herself. When the weather is nice, the puppies as a group are put in an outdoor pen.

Week 5

The mother is now only brought in to give milk. Puppies are fed in subgroups of 2-4 and when possible, solid food is introduced. Puppy transport boxes are brought into the room and left open so that the puppies can explore and sleep in them. Puppy activators and visitors, such as school children conducting community service, regularly enter the room, feed the pups, and play with them. In particular, the play fight "tug of war" is stimulated. More toys are introduced, which are noisier. When the weather is good, the puppies are taken outside in smaller groups and kept in pens or as a free-running group.

Week 6

The pups are given their first vaccination shots. They are named, and given a collar with their name on it. They are fed in subgroups of 2-3. Puppy activators intensify the play fight. Puppies start training at a low level; they are taken out in groups of 2 and introduced to different environments. Occasionally, they sleep in the transport boxes.

Week 7

The puppies are moved out of the litter box and breeding room and transferred to puppy kennels, where the temperature is no longer regulated. Groups containing 2-3 puppies are made, which allows for a smooth transition. They are fed together in their kennel group. The puppies are taken out separately by the puppy activator and introduced to multiple environments, including an obstacle course. The puppy activator uses a toy that encourages the dogs to chase it, thereby stimulating the puppies to move over different surfaces and obstacles. The puppies are taken out in a car to other environments. "Interaction training" is started, which involves calling the puppy by name and then rewarding attention with some food.

Week 8

The puppies are kept separately in puppy kennels. In case of good weather conditions, they are outside all day in puppy pens. The puppy activator continues to play tug-of-war games using a leather rug as a "big prey," and introduces simple hunt and/or search exercises using the same rug. The

pups are familiarized with walking on a leash, and the interaction training is continued by using food as a reward.

Week 9

Interaction training using food as a reward continues. Play and/or retrieve games are continued with the leather rug and with a Kong in different environments. Environmental training continues and after their final vaccination shots the pups are taken out in a car to different places in the city, such as a shopping centre.

Week 10

While the environmental, interaction, and search training continues, each dog is tested only on 1 morning in this 10th week.

Brief description of training program at GTC

The training at GTC produces MDDs that are capable of searching boxes with an area of 10×10 m^2 in a systematic manner to locate buried landmines. 'Long leash' search dogs work in a direction perpendicular to the handler, searching into the box in a straight line by moving away from the handler and then returning back. The handler makes the dog start at the left corner of the box, and systematically moves to the right, thus clearing the box (Figure 1). In contrast, 'short leash' search dogs work in a direction parallel to the handler, walking with him inside the box from the left to the right side of the box through the use of lanes and a "safe line." The handler then moves the safe line forward into the area of the box where the dog has searched, and a new lane is searched from left to right (Figure 2).

The training can be divided into the following 3 stages: primary training, intermediate training, and advanced training.

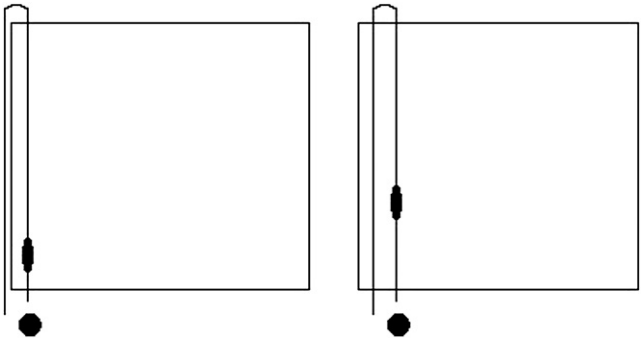

Figure 1 Long-leash search pattern, where the dog searches in a direction perpendicular to the handler and the handler stays in the safe line outside the 10×10 m^2 box. The dog moves to the end of the box away from the handler, turns left and returns back, thus completing the search lane. The handler systematically moves a small distance to the right after the dog returns so that the dog covers the whole area inside the box from left to right.

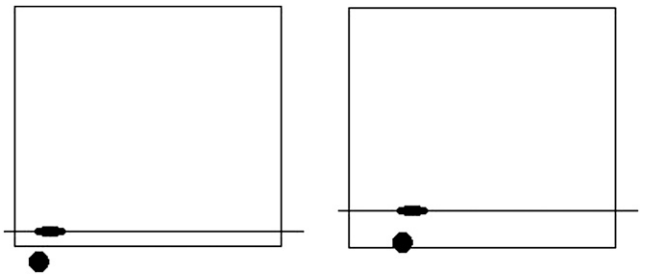

Figure 2 Short-leash search pattern, where the dog searches in a direction parallel to the handler in a single search lane from left to right. After each lane, the handler moves into the area of the 10×10 m^2 box that has been cleared by the dog so that the whole box is cleared from the front line to the back.

Primary training

The dogs are trained to detect increasingly smaller pieces of Kong that are hidden in/on objects such as cars and brick walls. They are stimulated to find the pieces without any help; usually a smaller and a larger piece are hidden to gradually increase the sensitivity of the dogs. The smallest pieces are like a grain of sand. The dogs (usually pups) are rewarded with a Kong when they really focus on the sliver of Kong and indicate the proper location of the Kong. The dogs must continue to look at the location that they have identified so as to receive their reward; a Kong is thrown over their heads to the place at which the sliver is hidden.

Intermediate training

After young dogs have the required search intensity for the sliver of Kong on/in different objects, direction training is initiated. For direction training, the dogs are first trained to conduct a long-leash search pattern, that is, the dog walks away from the handler and searches the ground in a straight line for a distance of 10 m, then makes a left turn (combined with the command "Kom sek") and comes back searching in a parallel straight lane. This search pattern is shaped initially by using wooden barriers and then later tape as guidelines. At first, the sniffing behavior pattern is shaped by using food on the ground. When the dog's search pattern and speed becomes consistent, the food is reduced and small pieces of Kong are introduced in the search lane.

The dog is then taught to properly indicate the location of the hidden object using "activity/passivity" training. In this training, the dog touches the Kong only when it is moving and not when it is lying still. At first, this training is conducted outside the search lanes, and it can be easily transferred to within the search lanes. Increasingly smaller pieces of Kong are provided in the search lanes and guidelines are diminished until there are none left.

In cases when a dog is not capable of conducting this long-leash search pattern reliably, it is transferred to the short-leash dog task. Dogs may also be assigned to short-leash searching when there is a larger operational requirement for short-leash dogs. A short-leash dog searches 10 m

lanes next to the handler, with the handler walking on the cleared area and the dog in the mine-suspected area. The search pattern is always from the left to the right side of the box. The same principles used in long-leash training with respect to rewards and "activity/passivity" training also apply to the short-leash search pattern.

Advanced training

In this phase of the training, the dogs are trained on mine odors. This is carried out by first training them to detect explosives and pieces of mine against different backgrounds in a carousel setup (Figure 3), where the explosives and mine fragments are initially coupled with pieces of Kong or the odor of Kong. The types of mines that are used in the carousel are determined by the types of mines the dogs will be confronted with in their future deployment. This part of the training is often conducted after the dogs have been transferred to the country from where they will be deployed. During the advanced training, the dogs may also be trained on "mine contamination," where a piece of explosive from a locally found mine is left on the grass for a few minutes and then removed. This results in a small contaminated area that can be used as a target for the training of the dogs. Finally, the dogs are trained in the training fields that are setup months before the arrival of the dogs. In these training fields, different types of mines are buried at different depths and securely mapped. Because the fields are left undisturbed for some months, the ground becomes completely stabilized and there are no disturbance cues left to which the dogs can respond.

Methods

The development of the puppies was monitored daily using a checklist with several developmental parameters such as opening eyes, reaction to sound, tail wagging, appearance of teeth, leaving the "nest," walking, and social parameters such as mounting another puppy, licking genitals, growling, barking, playing. Each litter was observed during 2 periods of 30 minutes daily. When a certain behavior was noted for a puppy within that timeframe, it was registered. Some developmental parameters were introduced later into the observations and were therefore not registered for all the puppies. By week 7, the observations ceased because the puppies were no longer in the litter.

Figure 3 Carousel with 24 arms used in the advanced training of mine detection dogs at GTC.

By week 10, all puppies were tested for their environmental skills and search behavior in a standardized test (Schoon, G. A. A. and Berntsen, G. A puppy test for mine detection dogs. Manuscript in preparation) by investigators who were blinded to the ENS condition of the pup being tested. In brief, the test involved placing each puppy in an unfamiliar room with an unfamiliar tester. The pup is then confronted with new and familiar objects that are first presented passively, and later moved around so that the pup can chase and play with them; distractions are introduced and the objects are suddenly removed to see whether the pup searches for them. The test was terminated immediately if the pup showed signs of distress at any time and its welfare was obviously at risk.

Puppies who passed this test and a vast majority who failed the test ($n = 74$) were taken into training. One puppy who passed the test was donated to another program. The dropout-rate and the scores of the dogs were monitored by the training staff. Two puppies died (1 at 13 weeks, the other at 25 weeks) during the training and testing period. The scoring data were incomplete as some dogs were transferred to Cambodia during their intermediate training and the trainers in that area used a different scoring method; however, the end result of the training of these dogs was noted. There were 3 possible end results: (1) Dogs developed into long-leash search dogs (this was the basic aim of the training); (2) When a dog failed to perform long-leash searching well enough, it was trained to be short-leash search dog; and (3) The dogs dropped out of the training and were donated to various other programs.

Results

The variation in the physical development of the puppies is illustrated in Table 2. Some parameters seem to develop with a higher variability, but this is probably an artifact as a result of the limited daily observation time. Mounting and licking of genitals did not occur often, thus the chance of this happening within an observation session is not very high. Other parameters that could be elicited, such as the reaction to sound, were much less variable. Statistical testing of the differences between the 2 groups revealed no significant differences in any of the parameters ($P > 0.05$, independent 2-sample t-test and independent 2-sample Wilcoxon signed-ranks test). No significant difference was found in the development of puppies experiencing

Table 2 The effect of ENS on studied developmental parameters

	ENS exercise group (n = 42)		Control group (n = 39)		
	Average (days)	Standard deviation	Average (days)	Standard deviation	One-sided *P*-value (*df*)
Day on which the first eye of pup opens	13.00	1.62	12.69	1.76	0.209 (79)
Pup reacts to soft sound (tapping on the side of the litter box) by turning its head toward the sound	16.14	1.77	15.81	1.22	0.184 (64)
Pup first wags its tail	21.12	2.61	20.87	2.73	0.389 (64)
Pup leaves "nest" and on its own finds a place to lay	27.09	8.94	27.32	6.51	0.452 (63)
Pup walks at least 1 m by itself	17.95	1.70	17.62	1.41	0.166 (79)
Day of appearance of first milk tooth	21.25	1.73	21.55	2.31	0.276 (67)
Day of appearance last milk tooth	33.38	2.51	33.71	2.98	0.318 (63)
Pup first eats semisolid food (porridge, yoghurt)	23.06	2.28	23.10	2.27	0.437 (63)
Pup first eats solid food (meat, dog biscuits)	44.06	7.71	44.10	7.60	0.492 (63)
Pup's first elimination (poo or pee) by itself without mother stimulating it	15.32	8.07	14.53	5.32	0.321 (62)
Pup first smells and/or licks its own genitals	31.63	5.71	32.60	7.56	0.325 (n = 14)*
Pup first smells and/or licks genitals of another puppy	28.50	7.53	27.00	8.64	0.365 (n = 16)*
Pup first mounts another pup	25.74	5.86	24.89	3.46	0.248 (56)
Pup first plays with a squeaky toy (that has been in the litter box the entire time)	35.55	4.11	35.18	4.07	0.375 (49)
Pup first barks	24.34	6.52	23.75	6.45	0.368 (54)
Pup first growls	25.47	6.34	25.78	6.20	0.425 (57)

The *P*-values for the difference between the parameters was calculated using the independent 2-sample *t*-tests.
*Parameters were analyzed by using the Wilcoxon signed-ranks test.

the ENS exercises and the control group with respect to any of the developmental parameters.

In Table 3, the effects of ENS on the puppy test are summarized. The difference in the pass rate of the 2 groups was not significant (χ^2 for independent samples, $P = 0.729$, $df = 1$). There seems to be no effect of the ENS exercises on the total test result, on test elements, or groups of elements.

In Table 4, the effect of ENS on the further training and final deployment of the dogs is described. Although more dogs with an ENS background were initially kept in training, their numbers quickly dropped because of poor training outcomes. Similar proportion of dogs with and without ENS graduated as MDDs (χ^2 for independent samples, $P = 0.881$, $df = 1$). There was also no difference in the level reached by the dogs (long- or short-leashed) (Fisher exact test, $P = 0.097$).

Discussion

This article presents unique data on the physical and behavioral development of puppies belonging to the pedigree Belgian Malinois during their first weeks of life, which can be used to compare development and outcomes of other breeds and other working dog programs. In our study, there was no effect of ENS on physical development, behavior during a test at 10 weeks, and later training results for puppies trained at GTC.

Table 3 The effect of ENS on the result of the puppy test

	ENS exercise group (n = 42)		Control group (n = 39)	
Total test pass	21 (50%)		21 (53.85%)	
Test terminated prematurely	4		4	
Average score on grouped test elements (maximum score = 4)	Average score	Standard deviation	Average score	Standard deviation
Environment and passive objects	3.24	0.81	3.25	0.84
First unfamiliar active object (ball rolling away)	2.92	1.09	2.90	1.05
Familiar active object (leather rug moved about)	3.54	0.72	3.65	0.61
Second unfamiliar active object (plastic bag moved about)	3.20	1.04	3.13	0.98

Table 4 The effect of ENS on the further training of young dogs and final deployment[a]

	ENS exercise group (n = 42)		Control group (n = 39)	
Dogs in training at	Number of dogs	Average score (maximum = 5); (±SD)	Number of dogs	Average score (maximum = 5), (±SD)
3 months	41	4.21 (±0.57)	33	4.28 (±0.53)
6 months	32	3.80 (±0.58)	26	4.13 (±0.55)
12 months	27	3.97 (±0.56)	22	4.25 (±0.58)
18 months	24	4.26 (±0.63)	21	4.50 (±0.41)
24 months	23	4.21 (±0.61)	20	4.56 (±0.48)
30 months	19	4.50 (±0.32)	19	4.61 (±0.44)
36 months	19	NA	18	NA
Final deployment	19 (45.2%)	9 long-leash and 10 short-leash	17 (43.6%)	13 long-leash and 4 short-leash

[a]The elements that are used to determine the score are search intensity, direction of search, and speed relative to where the dogs are in their training. Score 4 means the dogs are "on schedule" for their age/training level. When they are not performing well enough on any of the aforementioned points ≥ 1 points are subtracted. When they are performing better than expected for their age/training level, they are given a score of 5. Hitting the target and not giving false alerts are included in the elements that are judged only when the animals are about 18 months into their training.

In general, studies on the effect of postnatal handling during the early development stages usually reported some positive behavioral effects. For example, in the study conducted by Fox and Stelzner (1966), marked effects were found when comparing an extremely handled group with a severely socially deprived group. These effects were characterized as reduced anxiety and improved problem solving skills.

More recent studies have also found similar effects between less extreme groups. Gazzano et al. (2008) describe the effect of handling and kenneling, where "kenneling" meant that social contact was limited to daily cleaning until the pups were 8 weeks old, and "family raised" meant there were no restrictions on social contact. Handling meant "gentling," the pup is held in different positions by a trained person for about 5 minutes daily from days 3 to 21. Both handling and family-raising had positive behavioral effects, although these were limited to the latency to yelp in isolation (kennel-raised pups yelped at a later stage as compared with family–raised pups. Handled, kennel-raised pups yelped at a later stage as compared with nonhandled kennel-raised pups), the duration of this yelping (kennel-raised pups yelped for a shorter period), and the time spent exploring in isolation (handled pups spent more time exploring as compared with nonhandled pups in both situations). No differences were found in an arena test, where the pups were confronted with an unknown person and several different objects.

In a similar study by Sighieri et al. (2006), only the effect of handling ("gentling") was tested on puppies raised by professional breeders and families. However, no description was provided of the normal day-to-day routine of these puppies. In that study too, the only effect found was in the isolation test: with respect to latency to the first yelp, handled pups yelped at a later stage and spent more time exploring the novel environment. Also, no effect was found in an arena test. Still, both studies concluded that handling had sufficient positive effects—less discomfort in isolation and more well-being—for the authors to advise this treatment for puppies.

This advice is consistent with previous work carried out on rats. At the adult stage, postnatal handled rats show low anxiety-like behavior, which is expressed as high exploratory behavior and is correlated with low secretion of corticosterone in response to stress when compared with nonhandled controls (e.g., Vallée et al., 1997; Meerlo et al., 1999; Ábrahám and Kovács, 2001). A "bold" personality has been shown to be linked to the ability to learn and perform well in tasks requiring varied training in dogs (Svartberg, 2002).

ENS exercises certainly entail "handling" puppies. However, performing this particular set of exercises did not lead to any measurable effects in the development of the puppies at GTC or in their later success rates as MDDs. One possible explanation for this may well be within the extremely rich GTC socialization program. All puppies are handled daily and are systematically exposed to extensive environmental stimulation. In addition, even the control pups were held by a human being during exposure of the experimental pups to ENS, thus all the dogs experienced handling, thereby suggesting that both handling and exposure, and not specifically ENS, contribute to the outcomes of testing.

Performing ENS exercises minimally provides a structure for human contact with puppies, which is an important part of the socialization and exposure processes. This study showed that performing these particular exercises did not provide additional benefit in cases when the puppies are already being raised in socially and stimulatory rich environments. Larger, structured breeding programs for working dogs led by professional staff, such as guide dog programs or police dog programs, may provide such rich environments.

"Family" breeders who follow kennel club advice probably also provide sufficiently rich environments. For example, the Dutch "Raad van Beheer", the official pedigree authority in the Netherlands, provides breeders with the advice to handle puppies daily from the day they are born. The groups that are most likely to provide insufficient stimulation are "commercial" breeders who focus more on turnover rather than on the quality of puppies, including groups that breed dogs for research use, rescue or shelter organizations that may have limited resources, and working dog organizations with limited personnel and resources, and mandates that require production of extremely large numbers of dog. All these groups may benefit from the structure created from performing the ENS exercises with their pups.

Acknowledgments

The project was funded and approved by the Norwegian People's Aid, and conducted according to the ethical norms on animal treatment set by the management at the GTC. The authors thank Gordana Medunjanin for her daily observations of the puppies and her careful registration of their development.

References

Ábrahám, I.M., Kovács, K.J., 2001. Postnatal handling alters the activation of stress-related neuronal circuitries. Eur. J. Neurosci. 12, 3003-3013.

Battaglia, C., 2009. Periods of early development and the effects of stimulation and social experiences in the canine. J. Vet. Behav.: Clin. Appl. Res. 4, 203-210.

Fox, M.W., Stelzner, D., 1966. Behavioural effects of differential early experience in the dog. Anim. Behav. 14, 273-281.

Gazzano, A., Mariti, C., Notari, L., Sighieri, C., McBride, E.A., 2008. Effects of early gentling and early development of puppies. Appl. Anim. Behav. Sci. 110, 294-304.

Meerlo, P., Hovath, K.M., Nagy, G.M., Bohus, B., Koolhaas, J.M., 1999. The influence of postnatal handling on adult neuroendocrine and behavioural stress reactivity. J. Neuroendocrinol. 11, 925-933.

Scott, J.P., Fuller, J.L., 1965. Genetics and the social behavior of the dog. University of Chicago Press, Chicago, IL.

Seksel, K., Mazurski, E.J., Taylor, A., 1999. Puppy socialization programs: short and long term behavioural effects. Appl. Anim. Behav. Sci. 62, 335-349.

Sighieri, C., Mariti, C., Martelli, F., Baragli, P., Ducci, M., Gazzano, A., 2006. Effects of postnatal handling on the ontogenesis of canine behaviour. Vet. Res. Comm. 30 (suppl 1), 211-213.

Svartberg, K., 2002. Shyness-boldness predicts performance in working dogs. Appl. Anim. Behav. Sci. 79, 157-174.

Vallée, M., Mayo, W., Dellu, F., Le Moal, M., Simon, H., Maccari, S., 1997. Prenatal stress induces high anxiety and postnatal handling induces low anxiety in adult offspring: correlation with stress induced corticosterone secretion. J. Neurosci. 17, 2626-2636.