

A protocol for predicting performance in military working dogs: roles for anxiety assessment and genetic markers

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Abstract:

Many dog breeds exhibit what has been variously described as extreme “fear / shyness / nervousness / panic / anxiety” accompanied by social withdrawal. This condition is usually familial. There is a growing body of evidence suggesting that anxiety - at any level - can affect: (1) the rate at which learning progresses, and (2) various performance capabilities (King et al., 2000; Mills and Ledger, 2001). Such concerns are paramount for Department of Defense (DOD) military working dogs (MWDs) and Transportation Security Administration (TSA) dogs in the USA and analogous dogs elsewhere. MWDs and TSA dogs have never been more in demand or expected to work in as varied and complex environments as is the case today. MWDs are subjected to stressful and anxiety provoking situations during training and in the course of their work (Koda, 2001; Slabbert and Odendaal, 1999), which may be manifested as physical sequelae (Moore et al., 2001)

Prior work has indicated that a validated provocative test that assesses behavioral and physiological responses associated with anxiety in human adults is predictive of later anxious states in puppies from lines involving heritable forms of anxiety and panic (Overall and Dunham 2004 a,b). By coupling behavioral assessments - which unfortunately are not usually phenotypes - to physiological and behavioral phenotypes, and to genetic analysis of these phenotypes, we should be able to provide a probability analysis of puppies who as adults are likely to fail in either performance or testing measures. Genome scans have been successful in identifying simple Mendelian traits, and are finding increasing success in more complicated genetic disorders like anxiety-related behavioral conditions (Hamilton et al., 2003). Using a dense map of microsatellite DNA markers (Parker et al., 2004; Sutter and Ostrander, 2004), we expect to be able to map chromosomal regions in these dogs that contain genes related to the various performance, anxiety, and also olfactory behaviors that have genetic components. This will allow us to provide genetic counseling for preferred breeders based on the predictive power of the genetic markers that we have identified.

The use of molecular techniques allows us to move beyond quantitative techniques, where entire population means that may also affect other traits (eg, changing behavior also changes hip laxity) must be changed to get an effect. Here, individual probabilities for performance success and physical traits can be assigned to current and future breeders, so that the desired genes are collected in one set of breeding dogs. This approach adapts that used for assessing the genetic basis of disease states to selecting for suites of desirable behaviors, and then assessing those behaviors using new information theory, including neural nets.

Introduction:

Over 50 breeds across all 7 groups of AKC registered breeds of dogs have family lines in which extreme “fear / shyness / nervousness / panic / anxiety” is a major and often noted and breeder-reported concern (Overall, unpublished). Within these breeds this “trait” often follows familial lines suggesting a heritable basis. This pattern has been noted for many physical conditions in

dogs (Sutter et al., 2004), but little emphasis has been placed on behavioral conditions because of the difficulty in defining a clear phenotype. For 3 species in which anxiety has been investigated - dogs, humans, and mice - the more extreme the anxiety, the worse the ability to perform normal daily activities. Why care about this pattern if one is interested in any kind of working dogs?

In fact, the more extreme the performance demands, the greater the potential to provoke an anxious response. Studies in mice have shown that there are genetic lines of mice that are extraordinarily resistant to stress when asked to perform ever more complex tasks, and genetic lines of mice that are so susceptible to stress that they cannot learn even simple tasks (Yau et al., 1995, 2002). These experimental circumstances in mice have important applications for all working dogs. A review of the literature on canine social and cognitive behaviors will help make clear why anxiety-related behaviors are so important to consider when evaluating working dogs.

Canine social and cognitive systems:

Dogs share both foraging mode and a virtually identical social system with humans (Overall, 1997), and have co-evolved for co-operative work with humans for approximately 135,000 years, with intense selection for specific suites of behavioral traits (eg, the development of breeds) occurring in the last 12,000-15,000 years (Leonard et al., 2001; Vilà et al., 1997, 1999; Wayne and Vilà, 2001). Dogs mirror humans in hallmarks of social development (Overall, 2000). Recent data indicate that dogs are significantly more comparable to humans than are chimpanzees and wolves with regard to the complex social cognition involved in understanding long-distance signals that indicate where food is hidden (Hare and Tomasello, 1999; Hare et al., 1998, 2002; Miklósi et al., 2001; McKinely and Sambrook, 2000; Topál et al., 1997). Dogs are further able to communicate this information to other dogs (Hare and Tomasello, 1999; Hare et al., 2002; Pongracz et al., 2003). Also, like humans, dogs suffer from what we recognize as maladaptive anxiety - that which interferes with normal functioning - which was selected against during the co-evolution of dogs and humans.

Dog breeds were developed on the basis of specific work or jobs (e.g., border collies, Australian shepherds, Australian cattle dogs [herding]; Labrador retrievers [retrieving in water]; beagles [alerting for hidden prey]; Jack Russell terriers [tracking and killing small prey], Belgian Malinois [herding, guarding, and flock protection], et cetera). If breeds selected for different behaviors or jobs express different manifestations of anxiety when exhibited under extreme conditions, as is the case with working dogs, characterization of the response for different pedigree lines will provide information about variability of such conditions.

Finally, dogs may provide a fast-track for genome scans and mapping associated with factors affecting learning and cognitive function since breeds, by definition, are the result of canalized genetic variation. When a trait appears in a breed line it is likely that there is accompanying line breeding which can be identified by multi-generational pedigrees. It is not unusual to have 3 or 4 generations of dogs available for examination within any affected pedigree. Use of multi-generational dog families also allow us to examine individual differences instead of averaging across groups. Such analyses may better link structure and function than do analyses of groups (Plomin and Kosslyn, 2001).

Findings with respect to anxiety in dogs:

Anxiety disorders are among the most common health concerns in human medicine (Narrow et al., 2002), as they are for pet dogs (Overall, 1997). Furthermore, like humans, dogs with one anxiety-related diagnosis frequently have other anxiety-related diagnoses (Overall, 2000), suggesting the existence of some putative genetic or neurochemical liability. Although there are few quantitative clinical studies on anxious dogs, those focusing on separation anxiety (Overall

et al., 2001) and obsessive-compulsive disorder (OCD) (Overall and Dunham, 2002) have shown that a high percentage of affected dogs experience other, co-morbid anxiety disorders (~90% and 75%, respectively). In the case of separation anxiety, the co-morbid diagnosis is usually noise or thunderstorm phobia. While the data are few owing to the nature of retrospective studies, heightened noise reactivity or fear as a young dog may predispose the individual to the later development of separation anxiety. If so, this strongly suggests that associations between various anxiety and mood conditions (eg, depression and anxiety; panic and social phobias, etc.) may be the result of increased risk that is either the direct result of a shared underlying cause of the initial disorder, or the indirect result of neurochemical and, or molecular changes that occur because of the initial disorder. We have reason to expect anxiety that interferes with learning and behavioral plasticity follows the same general pattern (Knudson, 2004).

As is true for OCD, a large number of dog breeds contain family lines in which extreme “fear / shyness / nervousness / panic / anxiety”, involving social withdrawal, regularly occurs. Within these breeds the “anxiety” is found in multiple individuals within familial lines, appears to be heritable, and ranges from moderate shyness when faced with unfamiliar humans to intense avoidance and freezing behaviors. Four groups of laboratory dogs have also been described to have a variant of profound “anxiety”: ‘shy’ derivatives of crossbred Siberian huskies, derivatives of crossbred ‘nervous’ pointers (Klein et al., 1988; Overall et al., 1999), pure ‘nervous’ English pointers (Angel et al., 1982; Murphree, 1973; Murphree and Dykman, 1965; Murphree et al., 1967, 1971, 1974, 1977), and ‘anxious’ beagle-cocker crosses (Overall, unpublished). The labeling of these groups of dogs with different terms (eg, ‘shy’ v. ‘nervous’) is deliberate and reflects our lack of knowledge about whether these conditions are the same phenotypically or mechanistically. Behavioral terminology can be problematic and labels often have the effect of allowing us to think that we understand a phenomenon when we do not. This nomenclature also avoids the assumption that the aberrant behavior exhibited by one breed is isomorphic with that exhibited by another breed.

Findings with respect to learning and associations with anxiety:

There are few canine data on learning and anxiety. Canine aging is known to affect learning and various types of memory (Siwak et al., 2001; Tapp et al., 2003, 2004). There is evidence that treatment with monoamine re-uptake inhibitors speeds learning of specific tasks in dogs (Mills and Ledger, 2001). Similar results have been reported for mice for age-associated impairment in maze learning (Yau et al., 1995; Yau et al., 2002). The mechanisms postulated for these outcomes involve the finding that chronic glucocorticoid excess interferes with learning at the cellular level (Diamond et al., 1992; Pavlides et al., 1993). This chronic exposure has also been proposed to affect hippocampal neuronal structure (Sapolsky, 1996).

Viewed in this light chronic cortisol elevation may act as a translational gene regulator - a hormonal response element - in regions of the hippocampus. This finding is relevant for the large, but overwhelmingly non-experimental literature on working dogs: the single best predictor of failure in any working dog is fear, and the factor that prohibits most dogs from completing training programs is their fearful / anxious / uncertain response to novel or complex environments (Goddard and Beilharz, 1986; Hilliard and Burghardt, 2001; King et al., 2003; Slabbert and Odendaal, 1999; Svartberg, 2002).

Findings with respect to learning, anxiety, and olfactory responses:

Olfaction is the key sense by which detection dogs detect explosives (Gazit and Terkel, 2003a), and stimuli which provoke fear interfere with performance (King et al., 2003; Slabbert and Odendaal, 1999). Decrements in cognitive function that are associated with various neuropsychiatric conditions in humans (eg, schizophrenia, Parkinson’s disease, Alzheimer’s disease) are accompanied by changes in olfactory system neurons that may include density of

neurons, branching and connectivity of neurons, and changes in the ability of the neurons to maintain function associated with changes in RNA associated protein translation. Schizophrenics have smaller olfactory bulb volume than do control subjects (Turetsky et al., 2000) and olfactory epithelial neuron development becomes dysregulated in schizophrenia (Arnold et al., 1998, 2001). The olfactory dysfunction associated with degenerative neurological conditions that affect cognition includes deficits in odor detection threshold sensitivity, odor identification, and odor memory (Buck and Axel, 1991; Kratz et al., 2002; Laurent, 2002; Mombaert, 1999). Although the complete neurobiological basis of olfactory impairment in conditions that affect cognition is not known, each aspects known to be affected - odor detection threshold sensitivity, odor identification, and odor memory - is essential for MWDs and TSA dogs (Gazit and Terkel, 2003b). Furthermore, olfactory capabilities are impaired during illness and worsen with duration of illness (Arnold et al., 2001; Turetsky et al., 2000), and may be most affected with regional frontal lobe and basal ganglia impairment (O'Leary et al., 1999). These limbic regions are directly involved in both anxiety and the integration of conscious acts based on sensory stimuli.

Because olfactory system are the only neural tissues that continuously regenerate throughout life, we can potentially track olfactory function during the behavioral development and training of these dogs. Olfaction in dogs is poorly understood because it is complex. Odorant genes comprise the largest known family of G-coupled protein receptors (Buck and Axel, 1991; Kratz et al., 2002; Laurent, 2002; Mombaert, 1999). Odor reception occurs when odor ligands activate odor receptors in the olfactory epithelium (OE) (Kratz et al., 2002); each neuron may only express a single allele of a single odorant receptor (OR) gene, but the range of odorant to which any specific OR can bind establishes the range of odorants to which the cell responds. In dogs, these differences appear stable across breeds, despite the selection for behavioral variation between breeds (Issel-Tarver and Rine, 1996). This finding makes the issue of factors affecting individual capabilities at either the detection or the cognitive level even more important.

Effects at the behavioral level are clear for dogs: dogs detect explosives using olfaction, not vision (Gazit and Terkel, 2003b), but factors that impair their ability to focus on the substrate impair their ability to detect odors. Non-specific anxiety in dogs results in panting and pacing (Overall, 1997), and this behavior is associated with the first tier at which olfactory capability is impaired: dogs can either sniff or pant, but they cannot do both (Gazit and Terkel, 2003b). Established detection dogs who both pace and pant when faced with environmental stressors (eg, extreme temperatures) demonstrate increased duration of searches, decreased percent detection, and decreased sniffing frequency, resulting in decreased detection efficacy. It's expected that if the stressor is internal (eg, anxiety) and present throughout the early ontogenic stages susceptible dogs will 'learn' to be more anxious and display more anxiety-related behaviors during all subsequent behavioral testing and training and they will be less able to learn the appropriate tasks. Such effects should be lessened in genetic lines specifically derived from calm, stable dogs (eg, the Labrador retriever MWDs) and exaggerated in genetic lines where no such selection has been used and where heightened reactivity is desirable because of the dual-purpose use of the dogs (eg, the Belgian Malinois MWDs).

Application of specific concerns to sniffer and military working dogs:

Military Working Dogs (MWDs) are bred and trained for extreme and complex behaviors. The most common reason these dogs fail to succeed in such programs involves some aspect of anxiety, which appears to interfere with their ability to learn complex tasks and, or to adjust to changing environments and demands. Overly reactive / aggression, uncertain, or nervous / anxious dogs are rejected from such programs during various evaluations that occur as training progresses. Accordingly, emphasis has been placed on measuring, assessing, determining, and, or evaluating 'temperament'. Temperament evaluations have sought to understand how early

performance during training is linked to individual or clustered ‘temperamental measures’ in a way that is predictive of later performance, whether desirable (which dogs will succeed) or undesirable (which dogs will fail) (Hilliard and Burghardt, 2001; Svartberg, 2002; Svartberg and Forkman, 2002). Unfortunately, success has been modest.

Accordingly, any assay that can map behavioral or physiological measures early performance to changes that occur in ontogeny will enhance predictability. Unfortunately, neither behavioral nor physiological responses to provocative situations are well represented by either linear response models or by standard predictive measures. Some of the problem here may be due to the fact that assessments of behaviors are ‘soft’ and may not have high reliability or repeatability. Problematic data are concerns of any evaluation that uses (1) questionnaires based on someone’s impression of a behavior or (2) tools based on behavioral evaluations using scales or ranks. Simply, identification of targeted behaviors or behavioral suites, coupled with evaluations of intensity, frequency, and latency, are superior to other evaluation schemes and are required if molecular genetic approaches are to offer anything to working dogs.

Before assessment of response variables - and the associated response surface - can be made, the extent to which any true behavioral and physiological responses vary in any population must be determined in a way that can be repeated and used by other researchers. This process will define the ‘response surface’ for any ‘trait’ for an individual (Overall, 2005; See Figure 1), and the summation of these response surfaces will describe the potential outcomes for the population of breeding dogs. Coupled with a molecular genetics approach, one can determine the performance trajectory for working dog breeding stock and their future offspring. This approach also allows determination of whether familial aggregation of anxiety-related behaviors that may interfere with the recovery required for performance of complex tasks in changing situations is mediated by genetic factors. Use of a comparative approach where 2 breeds from very different selection backgrounds (eg, Labrador retrievers used to detect explosives for the TSA and Belgian Malinois used by the DoD) undergo discrete characterizing of behavioral and physiological phenotype increases the power of the test. This approach also allows us search for candidate genetic markers for canine versions of genes associated with successful and unsuccessful performance.

In this integrated approach tests that have been useful for performance evaluations in dogs or other species may yield valuable information about how working dogs may perform. Using the lactate provocation test we could define the behavioral and physiological phenotypes of, for example, TSA and MWD, and assess how anxiety affects performance throughout ontogeny. There are data showing that dogs that fail to perform as adults cannot be identified as puppies or juveniles using traditional methods.

Dogs that appear behaviorally normal as puppies will be recognizably affected with anxiety-related traits as discussed above that will interfere with successful performance within 2 years, the age of attainment of social maturity in dogs. Whether they succeed or fail in training and deployment is also known by this age. It is unlikely that dogs that exhibit undesirable behavior as adults and who fail to enter the working program are truly indistinguishable from puppies who later go on to successful performance because the anxiety-related behaviors that interfere with learning and stressful performance affect juvenile behaviors, when these age-specific behaviors are appropriately examined.

For example, in a colony of dogs with a heritable anxiety, an abnormal or extreme behavioral or physiological response to intravenous lactate at 8 weeks of age predicted which dogs would be affected by 10-12 months of age. There is no reason to assume that the anxiety-related factors that affect learning and performance later in life are not also active early in life. The key is to know how to evaluate early effects.

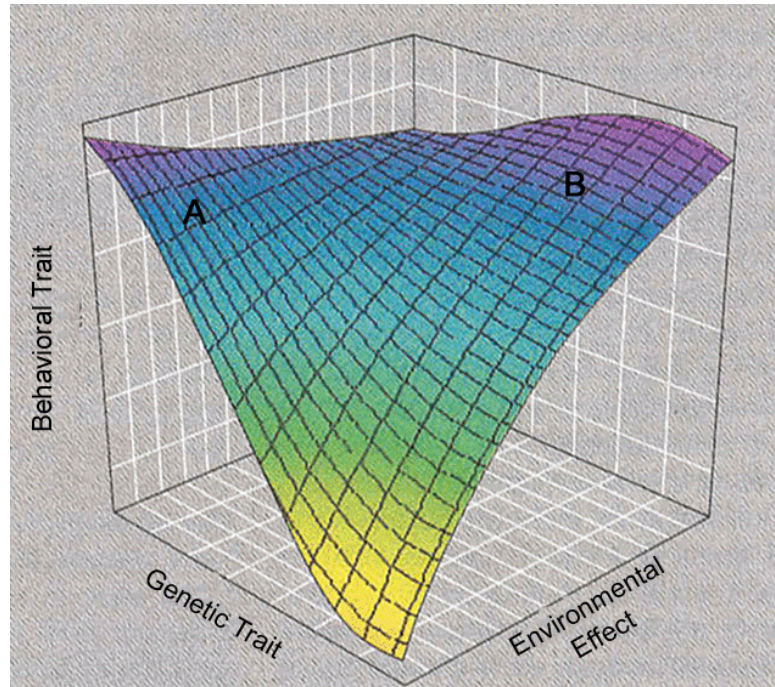


Figure 1: (From Overall, 2005, Figure 3): A model, complex, non-linear response surface that predicts what a trait or phenotype will look like given the effect of a certain gene and the effect of a certain environment. Note that at some points on this response surface the phenotype would be indistinguishable, even given wildly different environment and gene effect, whereas in other regions of the response surface a small environmental or genetic change can, alone, have a huge effect. This is the question we are always asking when we seek to understand temperament in dogs, for example: to what extent does the environment in which the dog lives display any genetic liability for any behavior? (Figure modified from Nijhout, 2003).

By comparing age-specific physiological and behavioral responses in 2 allied working breeds we can more accurately predict when genetic markers may be important. We propose that the level of anxiety-related behaviors at any ontogenic stage should be higher in Belgian Malinois than in the Labrador retrievers for the following reasons. (1) The Malinois are derived from a variety of European lines where enhanced reactivity has been preferentially selected. In contrast, the Labrador retrievers are derived from relatively few lines that experienced rigorous selection for a 'friendly temperament' and the ability to work calmly and steadily. (2) The Malinois are asked to be dual purpose [patrol and detection] MWDs and to perform more complex tasks than are the Labrador retrievers, and as such have been selected for heightened reactivity and responsiveness, rather than the calm, playful response selected for in the retrievers.

Preliminary data for a colony of purpose-bred dogs with heritable anxiety indicate that affected dogs respond to the provocative lactate test at an earlier age than that at which they exhibit their pathologically anxious behaviors. Accordingly, selection for a heightened responsiveness and reactivity should mean that successful and unsuccessful MWDs can be identified at an early age using the provocative test. Behavioral and physiological responses to this test should predict who will display an anxious phenotype that is incompatible with successful work and complex learning later. We would expect that at the same ages and stages in ontogeny, TSA retrievers should display a response surface different from that of the MWD Malinois, and that the outcomes predicted of later success or failure will also differ between breeds. This design allows

us to track neurodevelopmental components of both desirable and undesirable behaviors. By finely evaluating multiple generations on a regular basis, ontogenic changes in behavioral phenotype and the concomitant physiological response to infused lactate can be tracked by age / developmental stage. Clusters of behavioral patterns that are time penetrant should correlate with both the ontogenic progression of the phenotype and with otherwise non-specific responses to a provocative physiological test. Dogs that are behaviorally unaffected by anxiety-related learning and functioning constraints as adults should exhibit a range of physiological phenotypes during provocative testing, including some extremes that should be indicative of endophenotypes. Endophenotypes are defined as biological markers that define more homogenous, less variable, more similar subgroups that predict degrees of genetic association (Gottesman and Shields, 1972; Owen et al., 2004). The response of these endophenotypes should be informative - and possibly priceless - for breeding of working dogs.

By providing as complete a phenotypic description as possible for dogs of both breeds that exhibit ideal or preferred behaviors and those exhibiting undesirable or unacceptable behaviors, detailed categorical and quantitative phenotypic (behavioral and physiological) variation can be classified and later examined in the context of future first-pass genome scans. In this way we avoid discarding information that may be contained in patterns of how variation in behavior and physiology cluster. Furthermore, in addition to the behavioral analyses that are performed for behaviors exhibited during the provocative lactate test, there are 3 other potential behavioral assessments than can be added to any data base. (1) While subject to the concerns already discussed for all non-measured data, puppy walker assessments, especially if carefully logged onto tick sheets that encourage well defined assessment of key behavioral patterns, provide another source for some behavioral data. (2) The TSA and DoD in the USA conduct behaviorally provocative tests or evaluations throughout the first 18 months of the dogs' lives. Videotaping the dogs during these tests provides a unique opportunity to identify and quantify specific behaviors exhibited in response to certain stimuli, and also allows us to measure important variables such as latency to response, overall time spent attending to the stimulus, time to recovery, et cetera. It is this type of analysis that could be so useful, in combination with the data obtained during the lactate test, in identifying endophenotypes. (3) All dogs can also be re-tested using some subset of the routine behavioral tests immediately after undergoing the lactate test. If there are 'sub-clinical' effects of the test on some aspects of anxiety that affect performance, they may be most noticeable and best quantified post-test.

We can expand our predictions to include the realm of olfaction. As reviewed, there are now numerous studies linking anxiety and learning impairment or deficits. Panting and pacing are non-specific signs of canine anxiety. Dogs can either sniff or pant, but they cannot do both. It's expected that if the stressor is internal (eg, anxiety) and present throughout the early ontogenic stages susceptible dogs will 'learn' to be more anxious and display more anxiety-related behaviors during all subsequent behavioral testing and training, and affected dogs will be less able to learn the tasks at hand. Such effects should be lessened in genetic lines specifically derived from calm, stable dogs (eg, the TSA Labrador retrievers) and exaggerated in genetic lines where no such selection has been used and where heightened reactivity is desirable because of the dual-purpose use of the dogs (eg, the Belgian Malinois MWDs).

Successful graduation from training for all MWDs and TSA dogs depends on their innate olfactory capabilities, and their ability to accurately communicate locations of target odors. For these actions to take place both the olfactory system and the frontal cortex must act in an integrated way. Stress modifies the ability to work at peak performance and should affect olfactory firing. Olfactory integration - the cognitive, frontal lobe component of the olfaction task with which MWDs are faced - is subject to interference via the limbic system during periods of stress. Greater efficiency of olfactory abilities would be predicted for dogs who display less

anxiety on provocative tests. The Malinois who are cross-trained for detection and patrol should suffer greater consequences of early provocative anxiety because the tasks they are intended to perform are more complex.

Roles for heritability and genetic analyses:

Genome scans have been successful in identifying simple Mendelian traits, and are finding increasing success in more complicated genetic disorders like anxiety-related and other behavioral conditions. Using a dense map of microsatellite DNA markers (Parker et al., 2004) we expect to be able to map chromosomal regions in these dogs that contain genes related to the various performance, anxiety, and olfactory behaviors that have genetic components (Hamilton, 2005). What these mapped data mean for our ability to select for better overall dogs will depend on the quality of all of the other data available for assessment, validation of assessment methods - currently lacking, and on better methods for analyzing and interpreting high quality data.

New approaches to integrating data to assess predictability of outcome:

Assessing the effectiveness and predicting the outcome of different training and assessment protocols has proved challenging and poses serious problems for traditional methods of statistical modeling and analysis. For example, it would be valuable to accurately predict success or failure early in an evaluation and training sequence for potential working dogs. Accurate early categorization of candidates as successes or failures would save time and money and could lead to more reliable selection criteria. Questions such as these are not readily answered using standard statistical approaches. However, there are several relatively new approaches from the field of genetic programming which have demonstrated considerable promise in approaching these kinds of questions (Langdon and Poli, 2002).

One such method involves the construction of artificial neural networks (ANN) to approximate the complex relationship between a suite of input variables - like combined physiological, genetic, and behavioral assessments - and some outcome or output (Goh, 1995; Hastie et al., 2001). In the data used in its development and in the goals the technique aims to achieve, this technique is similar to many other multivariate techniques (Goh, 1995; Zell et al., 1995). In brief, an ANN consists of a network of interlinked nodes. The nodes and their linkages define the structure of the model. Usually, one or more layers of hidden nodes are sandwiched between an input layer and an output layer of nodes. The input nodes typically take the form of multivariate data (e.g., scores on behavioral tests), with each node corresponding to a different parameter. Values in the most commonly used form of ANNs are fed forward from the input layer into the hidden layer(s) and processed there. Each hidden node multiplies the input pattern by a series of connectivity weights. These values are summed and fed into a nonlinear activation function along with an additional value, known as the bias. The resulting values are then fed to the output node. Finally, the values from the hidden nodes are processed at the output node in a similar manner. The resulting output (e.g., prediction of success or failure in a behavioral training or assessment program) then compared with a known value (e.g., actual success or failure) to determine the accuracy of the output of the ANN model. ANNs are usually developed (trained) iteratively by presenting a network with a series of data the network is to recognize, quantifying the error between produced and actual output, and then adjusting the weights present at the hidden nodes through a variety of methods (Hastie et al., 2001).

Finally, the generality of the ANN is assessed through its performance on a test set or series of test sets not used during the training process. The strength of the neural network approach lies in the inherent nonlinearity or complexity of the functional relationships being analyzed (Scardi and Harding, 1999; Manel et al., 2000). This means that ANN can be used to determine the following:

1. when data and procedures used in repeated assessments are confounded with final

outcome decisions;

2. which scores are more tightly coupled to successful outcomes;
3. identification of outcome / assessment associations with failure early in the assessment scheme;
4. identification of trainers who are good or bad with respect to success rate;
5. identification of good candidates for cross-training;
6. identification of how large a 'success' effect will be;
7. identification of who will be the best breeders.

ANN models have advantages over general linear models in analyzing complex systems because they are capable of approximating complex nonlinear relationships, require no assumption of normality for the data, and exhibit better predictive success (Hastie et al., 2001; Lek and Guégan, 2000; King et al., 2000). ANNs are widely available in statistical packages (e.g. S-Plus and R - Venables and Ripley, 2002; JMP 5.1- SAS Inst, 2003).

References:

Angel C, DeLuca DC, Newton JEO, Reese WG. Assessment of pointer dog behavior. *Pavlov J Biol Sci* 1982;17:84-88.

Arnold SE, Han L-Y, Moberg PJ, Turetsky BI, Gur RE, Trojanowski PQ, Hahn C-G . Dysregulation of olfactory receptor neuron lineage in schizophrenia. *Arch Gen Psychiatry* 2001; 58:829-835.

Arnold SE, Smutzer GS, Tojanowski JQ, Moberg PJ. Cellular and molecular neuropathology of the olfactory epithelium and central olfactory pathways in Alzheimer's disease and schizophrenia. *Ann NY Acad Sci* 1998;855:763-773.

Buck L, Axel R. A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell* 1991;65:175-187.

Diamond DM, Bennett MC, Fleshner M, Rose GM. Inverted-U relationship between the level of peripheral corticosterone and the magnitude of hippocampal primed burst potentiation. *Hippocampus* 1992;2:421-430.

Dykman RA, Murphree IF, Ackerman PT. Litter patterns in the offspring of nervous and stable dogs. II. Autonomic and motor conditioning. *J Ner Ment Dis* 1966;141:419-432.

Gazit I, Terkel J. Explosives detection by sniffer dogs following strenuous physical activity. *Appl Anim Behav Sci* 2003a;81:149-161.

Gazit I, Terkel J. Domination of olfaction over vision in explosives detection by dogs. *Appl Anim Behav Sci* 2003b;81:65-73.

Geffen E, Gompper ME, Gittleman JL, Luh H-K, Macdonald DW, Wayne RK. Size, life-history traits, and social organization in the canidae: a reevaluation. *Am Nat* 1996;147:140-160.

Goddard ME, Beilharz RG. Early prediction of adult behaviour in potential guide dogs. *Appl Anim Behav Sci* 1986;15:247-260.

Goh, ATC. Back propagation neural networks for modeling complex systems. *Artificial Intelligence Engineering* 1995;9:143-151.

Gottesman II, Shields J. Schizophrenia and Genetics: A Twin Study Vantage Point. New York: Academic Press, 1972.

Hamilton SP. Mapping genes for anxiety disorders: humans as models for dogs. TVJ 2005;169:in press.

Hamilton SP, Fyer AJ, Durner M, Heiman GA, Baisre DL, Hodge SE, Knowles JA, Weissman MM. Further genetic evidence for panic disorder syndrome mapping to chromosome 13q. PNAS 2003;100:2550-2555.

Hare B, Tomasello M. Domestic dogs (*Canis familiaris*) use human and conspecific social cues to locate hidden food. J Comp Psychol 1999;113:173-177.

Hare B, Call J, Tomasello M. Communication of food location between human and dog (*Canis familiaris*). Evol Commun 1998;2:137-159.

Hare B, Brown M, Williamson C, Tomasello M. The domestication of social cognition in dogs. Science 2002;298:1634-1636.

Hastie, T., R. Tibishrani, and J. Freidman. The elements of statistical learning: Data mining, Inference and Prediction. Springer-Verlag, Berlin, Heidelberg, New York, 2001.

Hilliard S, Burghardt WF Jr. Development and validation of behavioral testing instruments for longitudinal study of military working puppies. Proceedings 2001 International Working Dog Breeding Conference, San Antonio TX (CD ROM), IWDBA, 2001.

Issel-Tarvier L, Rine J. Organization and expression of canine olfactory receptor genes. PNAS 1996;93:10897-10902.

King J, Simpson B, Overall KL et al. Treatment of separation anxiety in dogs with clomipramine. Results from a prospective, randomized, double-blinded, placebo-controlled clinical trial. J Appl Anim Behav Sci 2000;67:255-275.

King, SL, Bennet KP, List S. Modeling noncatastrophic individual tree mortality using logistic regression, neural networks, and support vector methods. Comp. Elec. Agric. 2000;27:401- 406.

King T, Hemsworth PH, Coleman. Fear of novel and startling stimuli in dogs. Appl Anim Behav Sci 2003;82:45-64.

Klein E, Steinberg SA, Weiss SRB, Matthews DM, Uhde TW. The relationship between genetic deafness and fear-related behaviors in nervous pointer dogs. Physiol Behav 1988;43:307-312.

Knudsen EI. Sensitive periods in the development of the brain and behavior. J Cog Neurosci 2004;16:1412-1425.

Koda N. Inappropriate behavior of potential guide dogs for the blind and coping with the behavior of human raisers. Appl Anim Behav Sci 2001;72:79-87.

Kratz E, Dugas JC, Ngai J. Odorant receptor gene regulation: implications from genomic organization. TRENDS Genetics 2002;18:29-34.

Langdon W B, Poli R. Foundations of Genetic Programming. Springer-Verlag, Berlin,

Heidelberg, New York, 2002.

Laurent G. Olfactory network dynamics and the coding of multidimensional signals. *Nature Rev Neurosci* 2002;3:884-895.

Lek S, Guégan JF. (eds.) *Artificial Neuronal Networks, Applications to Ecology and Evolution*. Springer-Verlag, Berlin, Heidelberg, New York, 2000.

Leonard JA, Wayne RK, Wheeler J, Valadez R, Guillen S, Vila C. Ancient DNA evidence for old world origin of new world dogs. *Science* 2002;298:1613-1616.

Manel S, Dias JM, Ormerod SJ. Comparing discriminant analysis, neural networks and logistic regression for predicting species distributions: a case study with a Himalayan river bird. *Ecological Modeling*. 1996;120:337- 347.

McKinley J, Sambrook TD. Use of human-given cues by domestic dogs (*Canis familiaris*) and horses (*Equus caballus*). *Anim Cognition* 2000;3:13-22.

Miklósi Á, Topál J, Csányi V. Looking back on dog evolution: the human connection. *Proceedings 2001 International Working Dog Breeding Conference, San Antonio TX (CD ROM), IWDBA, 2001.*

Mills D, Ledger R. The effects of oral selegiline hydrochloride on learning and training in the dog: a psychobiological interpretation. *Prog Neuro Psychopharmacol & Biol Psychiatr* 2001;25:1597-1613.

Mombaerts, P. Molecular biology of odorant receptors in vertebrates. *Ann Rev Neurosci* 1999;22:487-509.

Moore GE, Burkman KD, Carter MN, Peterson MR. Causes of death or reasons for euthanasia in military working dogs: 927 cases (1993-1996). *J Am Vet Med Assoc* 2001;219:209-214.

Murphree OD. Inheritance of human aversion and inactivity in two strains of pointer dogs. *Biol Psychiatry* 1973;7:23-29.

Murphree OD, Dykman RA. Litter patterns in the offspring of nervous and stable dogs. I: behavioral tests. *J Nerv Mental Dis* 1965;141:321-332.

Murphree OD, Dykman RA, Peters JE. Genetically determined abnormal behavior in dogs: results of behavioral tests. *Conditional Reflex* 1967;1:199-205.

Murphree OD, Peters JE, Dykman RA. Behavioral comparisons of nervous, stable, and crossbred pointers at ages 2, 3, 6, 9, and 12 months. *Conditional Reflex* 1971;6:91-100.

Murphree OD, DeLuca DC, Angel C. Psychopharmacologic facilitation of operant conditioning of genetically nervous catahoula and pointer dogs. *Pav J Biol Sci* 1974;9:17-24.

Murphree OD, Angel C, DeLuca DC, Newton JEO. Longitudinal studies of genetically nervous dogs. *Biol Psychiatry* 1977;12:573-576.

Narrow WE, Rae DS, Robins LN, Regier DA. Revised prevalence of mental disorders in the United States: using a clinical significance criterion to reconcile 2 surveys' estimates [comment].

Arch Gen Psych 2002;59:115-123.

O'Leary, DDM, Yates PA, McLaughlin T. Molecular development of sensory maps: representing sights and smells in the brain. *Cell* 1999;96:255-269.

Overall KL (1997): *Clinical Behavioral Medicine for Small Animals*, St. Louis: Mosby.

Overall KL. Advances in veterinary behavioral medicine: roadmap for the 21st century. *TVJ* 2005;169: in press.

Overall KL (2000): Dogs as "natural" models of human psychiatric disorders: assessing validity and understanding mechanism. *Prog Neuropsychopharmacol Biol Psychiatry* 24:727-276.

Overall KL, Dunham AE (2002): Clinical features and outcome in dogs and cats with obsessive-compulsive disorder: 126 cases (1989-2000). *J Am Vet Med Assoc* 221:1445-1452.

Overall KL, Dunham AE. Lactate provocation as a tool for assessing canine anxiety. Part 1. Clinical profiles of dogs with separation anxiety and noise phobia compared with non-remarkable dogs. *Biol Psychiatry* 2004a, ms.

Overall KL, Dunham AE. Lactate provocation as a tool for assessing canine anxiety. Part 2. Anxiety profiles of 2 colonies of genetically "shy" and "nervous" dogs with reference to canine models of human anxiety conditions. *Biol Psychiatry*, 2004b, ms.

Overall KL, Dunham AE, Frank D (2001): Frequency of nonspecific clinical signs in dogs with separation anxiety, thunderstorm phobia, and noise phobia, alone or in combination. *J Am Vet Med Assoc* 219:467-473.

Overall KL, Dunham AE, Acland G. Responses of genetically fearful dogs to the lactate test: Assessment of the test as a provocative index and application in mechanistic diagnoses. *World Congress on Psychiatric Genetics*, Monterey CA. *Molec Psychiatry* 1999;4:S125.

Owen MJ, O'Donovan MC, Gottesman II. Chapter 10: Schizophrenia. In: *Psychiatric Genetics and Genomics*, Eds. McGuffin P, Owen MJ, Gottesman II, Oxford University Press, NY, 2004:247-267.

Parker HG, Kim LV, Sutter NB, Carlson S, Lorentzen TD, Malek TB, Johnson GS, DeFrance HB, Ostrander EA, Kruglyak L. Genetic structure of the purebred domestic dog. *Science* 2004;304:1160-1164.

Pavlidis C, Watanabe Y, McEwen BS. Effects of glucocorticoids on hippocampal long-term potentiation. *Hippocampus* 1993;3:183-192.

Plomin R, Kosslyn SM. Genes, brain, and cognition [comment]. *Nat Neurosci* 2001;4:1153-1154.

Pongrasz P, Miklosi A, Kubinyi E, Topal J, Csanyi V. Interaction between individual experience and social learning in dogs. *Anim Behav* 2003;65:595-603.

Sapolsky RM. Stress, glucocorticoids, and damage to the nervous system: the current state of confusion. *Stress* 1996;1:1-19.

SAS Institute Inc. Cary, North Carolina, USA, 2003.

Scardi M, Harding LW, Jr. Developing an empirical model of phytoplankton primary production: a neural network case study. *Ecological Modeling* 1999;120:213- 223.

Siwak CT, Tapp PD, Milgram NW. Effect of age and level of cognitive function on spontaneous and exploratory behaviors in the beagle dog. *Learning & Memory* 2001;8:317-325.

Slabbert JM, Odendaal JSJ. Early prediction of adult police dog efficiency - a longitudinal study. *Appl Anim Behav Sci* 1999;64:269-288.

Sutter NB, Ostrander EA. Dog star rising: the canine genetic system. *Nature Reviews Genetics* 2004;5:900-910.

Sutter NB, Eberle MA, Parker HG, Pullar BJ, Kirkness EF, Kruglyak L, Ostrander E. Extensive and breed-specific linkage disequilibrium in *Canis familiaris*. *Genome Res* 2004;14:2388-2396.

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

Svartberg K, Forkman B. Personality traits in the domestic dog. *Appl Anim Behav Sci* 2002;79:133-155.

Tapp PD, Siwak CT, Estrada J, Head E, Muggenburg BA, Cotman CW, Milgram NW. Size and reversal learning in the beagle dog as a measure of executive function and inhibitory control in aging. *Learn Mem* 2003;10:64-73.

Tapp PD, Siwak CT, Head E, Cotman CW, Murphey H, Muggenburg BA, Ikeda-Douglas C, Milgram NW. Concept abstraction in the aging dog: development of a protocol using successive discrimination and size concept tasks. *Behav Brain Res* 2004;153:199-210.

Topál J, Miklósi A, Csányi V. Dog-human relationship affects problem solving behavior in dogs. *Anthrozoos* 1997;10:214-224.

Topál J, Miklósi A, Csányi V, Doka A. Attachment behavior in dogs (*Canis familiaris*): a new application of Ainsworth's (1969) strange situation test. *J Comp Psychol* 1998;112:219-229.

Turetsky BI, Moberg PJ, Yousem DM, Doty RL, Arnold SE, Gur RE. Reduced olfactory bulb volume in patients with schizophrenia. *Am J Psychiatry* 2000;157:828-830.

Venables, W. N. and B. D. Ripley. *Modern applied statistics with S*. 4th Ed., Springer-Verlag, Berlin, Heidelberg, New York, 2002.

Vila C, Savolainen P, Lamdonado JE, Amorim IR, Rice JE, Honeycutt RL, Crandall KA, Lundeberg J, Wayne RK. Multiple and ancient origins of the domestic dog. *Science* 1997;276:1687-1689.

Vilà C, Maldonado JE, Wayne RK. Phylogenetic relationships, evolution, and genetic diversity of the domestic dogs. *J Heredity* 1999;90:71-77.

Wayne RK, Vilà C. Phylogeny and origin of the domestic. In: *The Genetics of the Dog*, edited by A. Ruvinsky and J. Sæmpon, CABI International, New York, 2001:1-14.

Yau JLW, Olsson T, Morris RGM, Meaney MJ, Seckl JR. Glucocorticoids, hippocampal corticosteroid receptor gene expression and antidepressant treatment: relationship with spatial learning in young and aged rats. *Neuroscience* 1995;66:571-581.

Yau JLW, Noble J, Hibberd C, Rowe B, Meaney MJ, Morris RGM, Seckl JR. Chronic treatment with the antidepressant amitriptyline prevents impairments in water maze learning in aging rats. *J Neurosci* 2002;22:1436-1442.

Zell, A., G. Mamier, M. Vogt, N. Mache, R. Hubner, S. Doring, K-U. Hermann, T. Soye, M. Schmazl, T. Sommer, A. Hatzigeorgiou, D. Posselt, T. Screiner, B. Kett, G. Clemente and J. Weiland. SNNS Stuttgart Neural Network Simulator User Manual, Version 4.1. (<http://www-ra.informatik.uni-tuebingen.de/SNNS/UserManual/UserManual.html>), 1995.