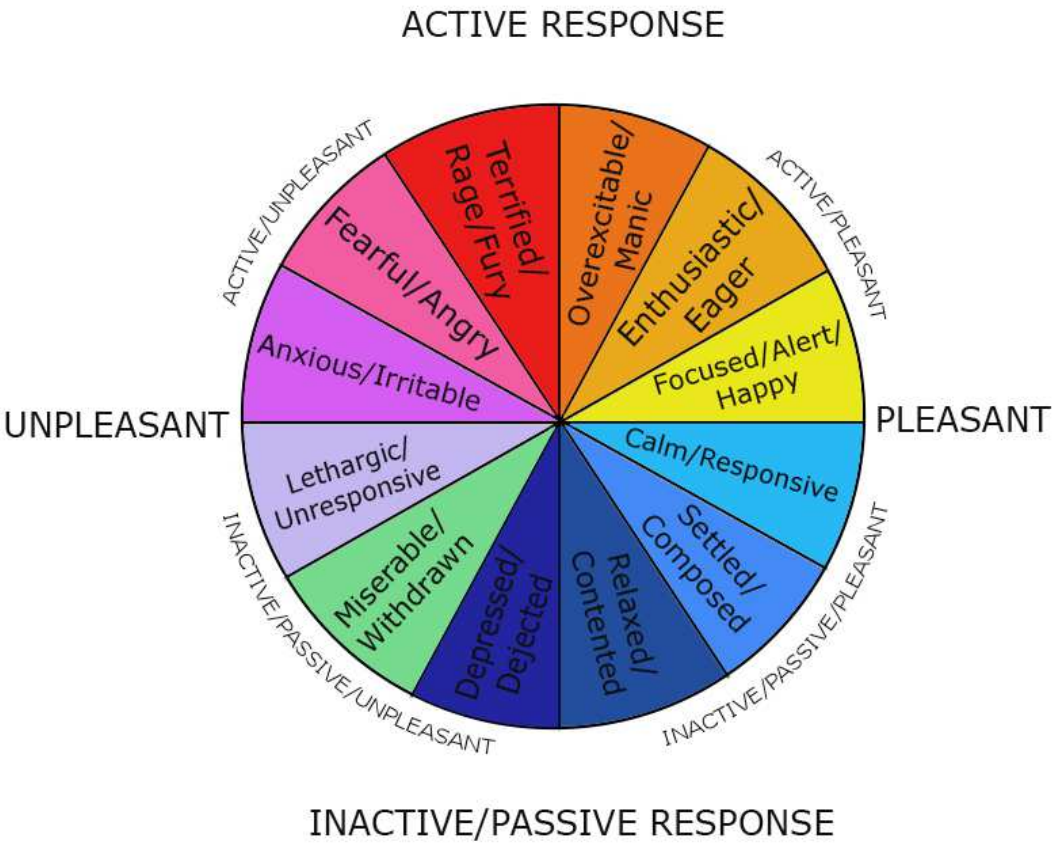

THE EFFECT OF DIETARY AMINO ACID CONTENT ON CANINE LEARNING AND BEHAVIOUR

Abstract

It has been shown in humans and other species that some amino acids in the diet directly influence brain activity by enhancing or reducing the rate of synthesis of different neurotransmitters. The level of tryptophan and other large neutral amino acids provided in the diet can influence the brain concentrations of these amino acids and subsequently alter behaviour. Mammals are unable to synthesise tryptophan. Therefore, levels in the brain depend on the presence of adequate dietary concentrations. Tryptophan is converted (in the terminals of certain neurons) into the indolamine serotonin, one of the monoamine neurotransmitters; the synthesis of serotonin has been shown to be influenced by the levels of the B-Group vitamins and insulin secretion. As serotonin has been shown to act as a mood stabiliser, and its deficiency has been implicated in learning difficulties, reinforcement contingencies and in a number of affective disorders, a diet which significantly increases levels of serotonin in the brain can have an important role in the treatment of canine behaviour and training problems.

Amino Acid **Tryptophan** **Serotonin Diet** **Canine-learning** **Behaviour**

Introduction

A leading cause of dog mortality is euthanasia due to behavioural problems. Well-meaning dog owners may adopt a pet with every intention of keeping the animal for its life span, but due to intolerable behaviour, are forced to terminate the relationship. Many owners simply do not have the time or resources to treat these behaviours through training or via professional help. A simple regimen that fits in well with the owner's daily schedule and results in improved learning capacity and mood stability can improve the dog's behaviour and maintain the pet/owner relationship.

Following on from the research carried out by Val Strong MSc, and the development of her serotonin enhancing diet, a dietary formulation that can help stabilise mood and emotional responses in dogs has been developed.

Background

Recently, several calming nutraceuticals have become available, but the use of these supplements

does not consider nutrient intake and absorption nor the metabolism of the individual animal.

Appropriate nutrition requires that all nutrients, carbohydrates, lipids, proteins, minerals, vitamins and water are ingested in adequate amounts and in the correct proportions. This is essential for normal organ development and function, reproduction, repair of body tissues and combating stress and disease. The nutrient intake must also be adjusted for varied levels of activity and physical work.

The design of an adequate diet depends upon two stages. The first is a knowledge of the nutrient requirements of the species in question (dogs), and the second is linking these to a given quantity in a collection of raw materials in a food (recipe). Nutrient requirements of animals are usually measured as daily amounts in relation to body weight. The energy content of the diet is determined by the energy supplying nutrients – protein, fat and carbohydrate. Energy is necessary for muscular work and to maintain body temperature. The first requirement for the dog from a diet is energy, and this is the principal factor governing the quantity of food needed each day.

The quantity of the food required by the dog will determine how much of each nutrient is taken in and introduces that important term **balanced diet**. A complete and balanced diet must contain all the necessary nutrients – protein, fat, minerals, vitamins and water (with carbohydrate and dietary fibre) under the following defined conditions:

- In the correct ratio to each other
- Within the optimum range
- In the correct ratio to the energy content of the food
- In a form which is usable by the animal

Furthermore, the food must be palatable so that eating is a pleasurable experience and the dog continues to enjoy eating it day after day.

Ingested protein is broken down into its component amino acids. It is well known that the amino acids tryptophan and tyrosine are converted to neurotransmitters in the mammalian brain. Tyrosine is converted to the catecholamine stimulants adrenaline, dopamine and noradrenaline, while tryptophan is converted to the indolamine serotonin.

Noradrenaline induces high states of arousal and has been implicated in the generation of aggression. Dopaminergic pathways in the brain are concentrated in the basal ganglia region and are involved in motor co-ordination, attention, reinforcement and reaction time. The indolamine serotonin was first discovered in 1948. Since then, a rather extensive network of serotonergic neurons has been identified in the mammalian brain which originate within the raphe-nuclei region situated in the brain stem.

At most synapses, serotonin produces inhibitory post-synaptic potentials, and its behavioural effects are also generally inhibitory [1]. Serotonin plays a role in the regulation of mood, the control of sleep and arousal, the regulation of pain and in the control of eating.

Low serotonin levels have also been implicated in alcoholism, obsessive compulsive disorders and other reward deficiency syndromes such as impulsivity, violent behaviour, suicidality, anti-social behaviour and attention deficit hyperactivity disorder [2][3].

It has been shown in humans and other species that certain amino acids directly influence brain activity and behaviour by enhancing or reducing the rate of synthesis of various neurotransmitters. The ratio of the concentration of the large neutral amino acids (tryptophan, tyrosine, leucine, isoleucine, valine and phenylalanine) in the diet can significantly affect the biosynthesis of the groups of neurotransmitters known as the Monoamines, which play a pivotal role in the regulation of arousal states.

The concentration of an amino acid in the diet or in the blood does not directly reflect its level in the brain. A complex group of blood-brain barrier mechanisms closely controls both the kinds of substances that enter the extracellular fluid of the brain and the rate at which they enter. Amino acids, amongst other important substrates, use an active transport mechanism, combining with transport proteins to cross the blood/brain barrier.

For amino acids, these carrier mechanisms are both size and charge specific. Within each carrier group, individual amino acids compete for uptake. Hence, an event such as meal ingestion can influence the level in the brain of a given amino acid by modifying its concentration in the blood and/or the blood concentration of other amino acids that compete with it for uptake. Therefore, the ratio of tyrosine or tryptophan to the sum of the other large neutral amino acids in the circulation will effectively control the amount of the amino acid taken across the blood-brain barrier.

The amount of tryptophan entering the brain depends primarily on the ratio of the plasma tryptophan concentration to the sum of the plasma concentrations of the other large neutral amino acids (leucine, isoleucine, valine, phenylalanine and tyrosine) [4][5].

This significant correlation between the serum levels of individual amino acids and their ratio to the sum of other transport competitors, supports the theory that competition between tryptophan and other large neutral amino acids is very important and is a dominant determinate of tryptophan uptake into the brain [2].

Tryptophan is present in relatively low amounts in high protein foods compared to other large neutral amino acids such as tyrosine. Therefore, when a meal which contains a high concentration of protein is ingested, tyrosine gains a competitive edge for entry into the brain. Conversely, following a high carbohydrate load, tryptophan enters the brain, although Fernstom and Fernstom [6] state that brain tryptophan can only be raised by carbohydrate intake if the carbohydrate meal is given within 2 to 3 hours of protein ingestion.

Serotonin in the brain is synthesised from tryptophan, but the rate-limiting step is hydroxylation of tryptophan to 5-hydroxytryptophan via tryptophan hydroxylase. This is then, in turn, converted to serotonin by aromatic L-amino acid decarboxylase [7]. Tryptophan hydroxylase is a low-affinity enzyme; a molecule of tryptophan and a molecule of the enzyme have no strong inclination to bind. Therefore, it is only when the concentration of tryptophan is much higher than normal that the enzyme can function at its maximum rate [8].

Amongst their other various functions, the B-Group vitamins maintain the functional integrity of the mammalian nervous system. The enzymes involved in serotonin synthesis are B6 and riboflavin dependent, as these act as co-factors, particularly in decarboxylation reactions. [5] As the B-Group vitamins are water soluble an adequate concentration needs to be provided in the diet daily.

Basis for Study

Val Strong MSc serotonin enhancing diet uses insulin, secreted in response to carbohydrate ingestion, to regulate plasma glucose levels and divert other large neutral amino acids to peripheral skeletal tissues where they are involved in energetic and immune system pathways - enabling tryptophan to gain a competitive edge across the blood brain barrier. Following demand from behaviourists and owners of problem dogs, Breakthrough has been developed to replicate this serotonin enhancing diet.

Of the 9.3 million dogs spread across 26% of households in the UK a significant number are either euthanised or relinquished to animal charities every year because of behaviour problems. Rescue kennels are stressful environments for most dogs and that stress can have a major impact on their welfare and management, and ultimately their chances of being adopted.

Animals have been selected to learn about reinforcing events [9]. However, if the brain's reinforcement mechanisms are impaired then the ability to experience reinforcing events will be reduced and learning affected accordingly. In fact, if an individual has a biochemical inability to derive reward from ordinary everyday activities then behavioural problems such as addictive, compulsive or impulse control disorders may result [10].

Dopamine is the primary neurotransmitter of reward in the limbic system, but at least 3 other neurotransmitters are known to be involved: serotonin, the enkephalins and gamma aminobutyric acid. ln a normal individual, these neurotransmitters work together in a cascade of excitation or inhibition leading to a feeling of well-being, the ultimate reward [11].

A disruption of these intracellular interactions results in anger, anxiety and other "negative feelings", or in a craving for substances which alleviate these negative emotions. Prolonged stress can lead to a self-sustaining pattern of abnormal cravings in both animals and humans. Research on alcoholic rats [12] shows that the increase of supply of serotonin at the synapse will reduce craving for alcohol. The biological substrates of reward are the basis for impulsive, compulsive and addictive disorders, comprising the reward deficiency

syndrome. The reward circuitry for habit forming behaviours is the same as that for natural rewards.

The reward cascade begins with the excitatory activity of serotonin releasing neurons in the hypothalamus. This then causes the release of the opioid peptide, met-enkephalin in the ventral tegmental area, which inhibits the activity of neurons that release the inhibitory neurotransmitter gamma amino butyric acid. The disinhibition of dopamine containing neurons in the ventral tegmental area allows them to release dopamine in the nucleus accumbens and in certain parts of the hippocampus, completing the cascade and mediating reinforcement effects.

Learning involves the strengthening of the connections between neural circuits that detect a stimulus and neural circuits that produce a particular response. An inability to experience reinforcing events, be they positive or negative, will lead to reduced learning capabilities and associated behaviour problems. A combination of genetic and environmental factors can affect an animal's ability to cope with novel situations and learning tasks [13]. The secretion of hormones, such as the corticosteroids, through dysregulation of the hypothalamic pituitary adrenal axis, in response to prolonged stress, can reduce brain levels of serotonin [14] explaining the many abnormal behaviour patterns sometimes seen in dogs in rescue shelters [15].

Serotonin, gamma amino butyric acid, glutamate, dopamine and opioid systems have been shown to be involved in mediating positive reinforcement systems [14]. A deficiency in the reward cascade causing lowered levels of serotonin can lead to negative states, such as depressed mood, dysphoria, irritability, and impulsive behaviour via a reduction of the behavioural inhibition system during learning [1].

Materials and Methods

The study was carried out at **Wood Green**, The Animals Charity, King's Bush Farm, Godmanchester.

All human and animal behaviour is influenced by moods and emotions, both of which affect each other [6].

Emotions involve a relationship between the individual and an explicit cause/object/event. Emotions are acute, and short-lived, lasting milliseconds, or minutes at most [17]. The cause that elicits an emotion (the stimulus) can be anything from an event in the environment to individual thoughts and memories [17].

In contrast, moods last for hours or even for several days [18]. Moods are not directed at a particular object or cause, but rather are a culmination of similar experiences. Consequently, we are generally unable to specify the cause of a particular mood [17].

Mood is the background feeling of the day, how the animal feels and behaves generally when not displaying the problem behaviour. A depressed dog will be much more difficult to motivate, and an overexcited dog is unlikely to be able to learn to focus and be calm when meeting people.

Behaviour problems are not diseases. Successful treatment depends upon accurate assessments of how the animal feels (i.e. fearful, angry, etc.) at the time the problem behaviour occurs and the animal's general mood state.

<u>Assessment tools</u>

When describing emotional states, the term often used is 'affect', meaning the experience of feeling or emotion. In 1988, Watson, Clark and Tellegen [19] published a paper introducing the Positive and Negative Affect Schedule (PANAS), based on the idea that positive and negative affect should be separately tracked because they vary independently.

Using the PANAS model, emotional states can be plotted on a two- dimensional grid. On the x-axis "pleasantness/unpleasantness," on the y-axis "arousal," or "activation" and emotional states are placed around these axes (Figure 1).

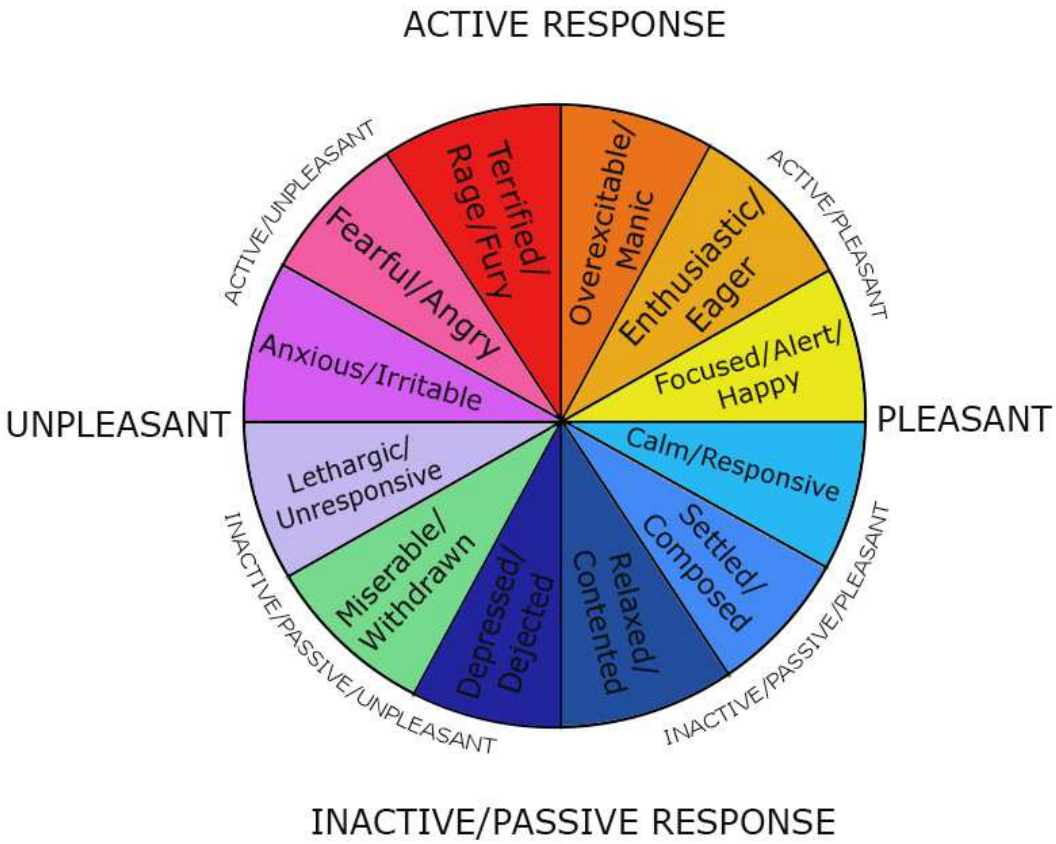

Figure 1

Active-pleasant emotional states are those of excitement and pleasurable engagement, while misery and lethargy are the opposite passive-unpleasant states. The active-unpleasant emotional states are those of distress or irritation and un-pleasurable engagement and include anxiety, fear, and terror as well as irritability, anger and rage. Passive-pleasant states are those of calmness and contentment.

Inability to express or engage in innate motor patterns may result in a lowered mood state, provoking intense emotional responses to various stimuli. Irrelevant behaviour patterns, displacement activities or coping strategies such as scratching or barking, may be exhibited in response to the frustration of intermittent reinforcement [15]. Inadequate or ineffective reinforcement may elicit adjunctive behaviours such as drinking or redirected aggression [20] or a hyperactivity state caused by the arousal of the noradrenergic pathways. Low

levels of serotonin have been associated with high locomotor responses especially in response to novel situations such as change in surroundings such as in a rescue shelter [12].

The assessment of emotional state/response provided an accurate measure of behavioural response within the kennel environments. Scores were given to all relevant emotional states to provide a validated scale of assessment. Scores of 1 to 10 indicated the observer's objective evaluation of the intensity of the dog's emotional state (Table 1).

Emotional Assessment

Please Tick:

10 being an intense negative emotional state and low positive emotional state

1 being an intense positive emotional state and low negative emotional state

Anxiety/ Agitated/ Unable to settle	10	9	8	7	6	5	4	3	2	1	**Calm/ Relaxed**
Before											
Week 1											
Week 2											
Week 3											
Reactivity Low impulse control	10	9	8	7	6	5	4	3	2	1	**Compose Responsi**
Before											
Week 1											
Week 2											
Week 3											

Table 1

Subjects

38 dogs of various breeds/type were included in the study. Average time in kennels was 58 days.

Inclusion into the study required the dogs to be in good health, no history of dietary intolerance, not receiving any veterinary treatment or medication, or any remedial behaviour therapy.

Two aspects of common problem behaviour within a kennel environment were studied:

Group1 (24 dogs)

anxiety/agitation/inability to settle **versus** calm/relaxed

Group 2 (14 dogs)

reactivity/low impulse control **versus** composed/responsive

Behaviour was assessed prior to entering the study (week 0), and at week 1, 2 and 3 of the study period.

Results

Although not one of our outcome measures, several dogs were able to be moved into the rehoming kennels part way through the trial.

GROUP 1 – 24 dogs

Dog 23 made no improvement on the diet.

In the remaining 23 dogs, there was an average 53% reduction in anxiety and agitation with a comparable 61% improvement in calm/relaxed behaviour.

GROUP 2 – 14 Dogs

In all 14 dogs, there was an average 55% reduction in reactivity with a comparable 59% improvement in composed/responsive behaviour.

Discussion

Rescue kennels are stressful environments for most dogs and that stress can have a major impact on their welfare and management, and ultimately their chances of being adopted.

These preliminary results clearly suggest that the diet had a significant positive effect on the dogs' mood states and emotional responses and their ability to respond to training.

Our results indicate that this diet formulation will have a profound effect on the welfare and management of dogs in rescue shelters and their successful rehoming.

The diet could improve the efficiency of behavioural therapy and potentially prevent some dogs being relinquished to rescue centres or euthanized.

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