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Short communication

Development of a minimally-invasive protocol for recording mismatch negativity (MMN) in the dog (*Canis familiaris*) using electroencephalography (EEG)

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ABSTRACT

Mismatch negativity (MMN), observed in event-related potentials (ERPs), constitutes a measurable change in electrophysiological brain activity occurring after exposure to a novel stimulus. In humans, MMN is considered to be related to stimulus discrimination at the cortical level. ERP recording in dogs may present an opportunity to increase understanding of cognitive processes without reliance on observable behaviour, which may be confounded by motivation or training. Preliminary data are presented suggesting the existence of MMN, recorded using a minimally-invasive procedure equivalent to that used in humans, in unrestrained, unanaesthetised dogs. This is the first example of this ERP component in dogs and the method has substantial utility for future research exploring auditory, olfactory, and visual discrimination tasks, development, and breed differences.

1. Introduction

Dog cognition research has experienced an abrupt revival in the past decade (Wynne, 2009).

Currently, a dog's behaviour is the only mechanism for establishing whether a dog has the cognitive capacity to perform a given task but, in some situations, behaviour may be confounded by other factors, such as obedience training, motivation levels, and prior experiences. A tool that indexes dogs' cognitive abilities in the absence of a behavioural response is needed. Innovation in the use of EEG might allow researchers to answer some important questions that are currently unresolved.

Within an EEG, an event-related potential (ERP) measures a change in brain activity after exposure to a stimulus (Luck, 2005). The MMN potential is a negative-going component of the ERP waveform that is observable after exposure to an unexpected stimulus (Luck, 2005). In auditory paradigms, when embedded in a series of identical tones, a novel tone of different pitch, volume, duration, or some other stimulus property, may elicit this waveform. In addition to auditory research, MMN can be elicited by stimuli that evoke a response from visual and olfactory senses (Naatanen et al., 2007). MMN has been implicated in language processing in humans, and research examining MMN has been studied in Alzheimer's patients (Naatanen et al., 2007). It can be elicited when the subject is not explicitly focused on the tones, and is believed to be related to an automatic switch of attention from pre-attentive echoic sensory memory (Naatanen et al., 2007). Because the MMN waveform is considered to reflect higher-order cognitive processing related to memory, but does not require focused attention, it could be particularly useful in discrimination tasks with dogs.

MMN has been demonstrated in cats (Pincze et al., 2001), rats (Ruusuvirta et al., 1998), and monkeys (Javitt et al., 1996), all of which show MMN responses to deviant stimuli. MMN has not been reported in dogs, although a study of ten 15 week old puppies did report an ERP response (Adams et al., 1987). This suggests that dogs may exhibit MMN ERP's, which would give dog cognition researchers a mechanism other than behavioural response for studying stimulus discrimination in dogs. However, the study by Adams et al. (1987) was conducted while the puppies were sedated. This is problematic for dog cognition research, as are the invasive procedures used previously in other animal species. These have included resection of skull portions (Javitt et al., 1996), and/or placement of electrodes on the dura mater (Pincze et al., 2001).

Such techniques are unsuitable for dog cognition research for two reasons. First, anaesthesia or sedation is inadvisable, since these can affect cognitive processing of the stimulus (Koelsch et al., 2006). Second, there is general agreement among researchers that this type of research should be non-invasive whenever possible.

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This is in accordance with National Research Council policies on animal use in research (Guide for the care and use of laboratory animals, 2011) and also reflects the fact that researchers in dog cognition often work with pet dogs, recruited from the community. Many dog owners consider their pets to be members of the family (Miklosi, 2008) and access to these animals requires that the dogs leave the study area in the same physical and affective states as when they arrived; risking even mild pain or distress is unacceptable. In order to be a viable method for studying cognitive processing in dogs, ERP research needs to be conducted with the same level of concern for the subject as is the case in human research: being non- or minimally-invasive and painless. This is made difficult by the fact that dogs have much more hair covering their scalps than do humans, and a thick skin that raises the impedance level to more than 50 kΩ.

Although some EEG research with animals has employed traditional, invasive, methods, other studies have tried to adapt less invasive methods for use in dogs (Greene et al., 1992). One recent study from the field of epilepsy research, for example, aimed to standardise placement of small, subdermal needle electrodes in dogs with different shaped heads (Pellegrino and Sica, 2004). Subdermal needle electrodes are very small, approximately 10–15 mm in length, with a 27–30 gauge width; this makes them comparable in diameter to acupuncture needles (Ceniceros and Brown, 1998). The needles are inserted subcutaneously and do not need to penetrate the skull or muscle underneath the skin. Once underneath the skin, the impedance level drops from more than 50 kΩ to less than 5 kΩ, enabling accurate EEG recording. In an earlier study using subdermal needle electrodes in dogs, the electrodes were placed under the dog's skin while the dog was under anaesthesia (Greene et al., 1992), remaining in place throughout the duration of the recording and easily removed upon completion. Insertion of fine needles such as this should not be associated with pain or distress, making this technique a suitable candidate for use in non-sedated pet dogs. The aim of the current study was to develop a method for recording endogenous ERPs, such as MMN, from dogs using this minimally-invasive technique and without the use of any anaesthesia or pharmaceutical sedation.

2. Methods

This pilot study was approved by Monash University School of Psychology and Psychiatry Animal Ethics Committee: 2010/01-S1.

2.1. Participant

Our pilot subject was an 8 year old female Australian Shepherd, named Jaffa. The dog was recruited through word-of-mouth and selected on the basis of owner reports that she would relax quickly in novel environments, be obedient to owner commands of 'sit' and 'stay' for up to 10 min, and not be distressed when needles were inserted under the skin. Observations of the dog's behaviour both before and during the testing phase indicated that she could hear the auditory stimuli.

2.2. Materials

Testing was performed in a sound-attenuated laboratory at Monash University, Clayton Campus. Viasys Healthcare Disposable Rapid-Pull 12 mm Subdermal Needle Electrodes with 2.5 m cable (San Diego, CA, USA) were attached to a wire connecting the EEG amplifier. These electrodes are 27 gauge needles made of stainless steel. A high-grade D/C amplifier, Compumedics Neuroscan Synamps^2 70-channel (Charlotte, NC, USA), was used to record the dog's EEG. Stimuli were presented using Compumedics Neuroscan Stim2 (Charlotte, NC, USA) and the EEG was recorded using Compumedics Neuroscan Scan 4.5 (Charlotte, NC, USA). Data were analysed using Compumedics Neuroscan Scan 4.5 and MS Excel.

2.3. Procedure

2.3.1. Relaxation protocol

When the owner and dog arrived at the laboratory, the dog's collar and leash were removed, and the animal permitted to meet the experimenters and explore the laboratory until she appeared ready to settle on a dog bed next to the EEG amplifier. Although pharmaceutical sedation was unnecessary to obtain an accurate EEG recording, it was important that the animal be very relaxed. Therefore, the owner remained present at all times and full relaxation took approximately 40 min. This was determined based on behaviour; Jaffa was deemed relaxed and ready for electrode placement when she lay on the bed, with her head on the floor and eyes closed, for approximately 5 min without attempting to leave the bed, changing position, or fidgeting.

2.3.2. Needle electrode placement

When the dog was fully relaxed, electrode placement commenced. The recording electrode was placed at Cz, on the midline 50% of the distance from the stop (the indentation of the bone between the eyes) to the external occipital protuberance. This was selected because human studies often show a large MMN response at midline sites (Luck, 2005), and it is simple to identify, requiring measurement of the distance between two easily visible points on the animal's head. In humans, Cz corresponds to the central sulcus; this is analogous to the canine cruciate sulcus which is located rostral to our Cz placement in dogs (Pellegrino and Sica, 2004). Since MMN can be recorded throughout midline sites in humans, we anticipated that it would be possible to accurately record MMN on the midline in a dog even though the corresponding areas of the brain may differ somewhat.

Because EEG measures electrical impulses occurring in the brain, a reference electrode is necessary to compare electrical activity in the brain to another part of the body. It is important that the reference site has as little electrical activity as possible, and the mastoid or earlobe is commonly used in human research (Luck, 2005). Because the dogs' head has muscles throughout the mastoid and skull (Smith, 1999), this placement was expected to produce too much muscular interference on the EEG recording. Therefore, a midline site was chosen on our pilot subject's neck, 100% of the distance from the stop to the external occipital protuberance, starting from Cz.

A third electrode was necessary to close the electrical loop within the dog's body to reduce electrical interference from outside the body. This ground electrode was placed at Oz, which is 10% of the distance from the stop to the external occipital protuberance, rostral to the external occipital protuberance. Therefore, in this dog, the recording electrode was placed at Cz, 6 cm behind the stop, the reference was placed 12 cm behind Cz on the neck midline, and the ground was placed 1.2 cm rostral to the external occipital protuberance. These three electrodes were the minimum required to record EEG using the Synamps^2 system. See Fig. 1 for illustration of the placement.

The needle electrodes were inserted underneath the skin and were secured with surgical tape. At no time did the dog show any signs of pain or distress upon insertion; in fact she gave no behavioural indication that she had even noticed their insertion at all. Once the recording was complete, the tape and electrodes were removed and disposed of.

2.3.3. Stimulus presentation

Once the animal was fitted with the three needle electrodes, an auditory oddball paradigm was run using 50 ms tones. The