CHAPTER 3

EVOKE POTENTIALS

3.1 INTRODUCTION

A signal is defined as single valued function of one or more independent variables that conveys some useful information about the state or behavior of a physical system [53]. Signals can be one dimensional, if they depend on a single variable such as time, or multidimensional if they depend on several variable such as spatial coordinates.

The function of human body is associated with signals of electrical, chemical, or acoustic origin. Such signals convey information which may not be immediately perceived but hidden in the signal structure. This information has to be extracted and decoded to give the signals some meaningful interpretations. These signals reflect properties of their associated underlying biological systems, and their extraction has been found to be very useful in the diagnosis of many diseases. The extraction process is sometimes straightforward and may involve very limited manual effort such as visual inspection of the signal on a paper printout or computer screen. However, the complexity of a signal is often quite considerable, and therefore biomedical signal processing has become an indispensable tool for extracting clinically significant information hidden in the signal.

3.2 BIOMEDICAL SIGNALS

A signal is said to be biological if it is recorded from a living being. Some examples of temporal signals are temperature record of a patient, voltage recorded by an electrode placed on the surface of the body or scalp, etc. Two dimensional spatial signals include images, X-ray, CT or MRI scan impressions, etc. Biomedical signals are functions of time obtained from human physiological system. These signals also
depend on the spatial position on the body of the subject from where the signal is acquired. They are the records of the electrical, chemical or mechanical activities that occur during biological events, such as human heart beat or muscle contraction. Biomedical signals provide information about the state of health of the biological system that produces these signals.

An electrocardiogram (ECG) describes the electrical activity of the heart recorded by electrodes placed on the body surface. The voltage variations measured by the electrodes are caused by the action potentials of the excitable cardiac cells as they make the cells contract. The resulting heartbeat in the ECG is manifested by a series of waves whose morphology and timing convey information which is used for diagnosing diseases that are reflected by disturbances of the heart's electrical activity.

Table 3.1 Frequency range and amplitude of some typical biomedical signals

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Signal</th>
<th>Frequency (Hz)</th>
<th>Amplitude range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Electroencephalogram</td>
<td>0.2-50</td>
<td>600 µV</td>
</tr>
<tr>
<td>2</td>
<td>Electroculogram</td>
<td>0.2-15</td>
<td>10 mV</td>
</tr>
<tr>
<td>3</td>
<td>Electrocardiogram</td>
<td>0.15-150</td>
<td>10 mV</td>
</tr>
<tr>
<td>4</td>
<td>Electromyogram</td>
<td>20-8000</td>
<td>10 mV</td>
</tr>
<tr>
<td>5</td>
<td>Blood Pressure</td>
<td>0-60</td>
<td>400 mm Hg</td>
</tr>
<tr>
<td>6</td>
<td>Spirogram</td>
<td>0-40</td>
<td>10 L</td>
</tr>
<tr>
<td>7</td>
<td>Phonocardiogram</td>
<td>5-2000</td>
<td>80 dB</td>
</tr>
</tbody>
</table>

3.3 ELECTROENCEPHALOGRAM

The electroencephalogram (EEG) reflects the electrical activity of the brain as recorded by placing several electrodes on the scalp. The EEG is widely used for diagnostic evaluation of various brain disorders such as determining the type and location of the activity observed during an epileptic seizure or for studying sleep
disorders. The brain activity may also be recorded during surgery by attaching the electrodes directly to the uncovered brain surface; the resulting invasive recording is named an electrocorticogram (ECoG). The signals obtained from different locations on brain have different shape because of the variation in the oscillating behavior of neurons in different paths [54]. Table 3.1 shows normal values of frequency range and amplitude levels of some typical biomedical signals.

### 3.4 EVOKED POTENTIALS

Evoked potentials (EPs) constitute an event-related activity which occurs as the electrical response from the brain or the brainstem to various types of sensory stimulation of nervous tissues; auditory and visual stimulation are commonly used [1]. The recording of such electrical potentials represents a noninvasive objective test which provides information on sensory pathways abnormalities, the localization of lesions affecting the sensory pathways, disorders related to language and speech, etc.

![Image of brain showing sensory points related to different stimuli.](image)

Evoked potentials are recorded from the scalp using an electrode configuration similar to that of an EEG recording. The potentials typically manifest themselves as a
transient waveform whose morphology depends on the type and strength of the stimulus and the electrode positions on the scalp. The mental state of the subject, exemplified by attention, wakefulness, and expectation, also influences the waveform morphology. Fig. 3.1 shows the image of brain indicating sensory points related to different stimuli, such as visual, auditory and somatosensory stimuli. Fig. 3.2 shows placement of electrodes on the brain surface for recording multichannel evoked potentials.

![Fig. 3.2. Placement of electrodes to record multi-channel evoked potentials.](image)

Individual EPs have very low amplitude, ranging from 0.1 to 10 µV, and are hidden in the ongoing EEG background having an amplitude on the order of 10 to 100 µV. EEG in the present work is viewed as "noise" whose influence should be minimized so that the EP waveform can be subjected to reliable scrutiny. As a result, noise reduction is one of the most frequently addressed signal processing issues in the analysis of EPs. Fortunately, an EP usually occurs after a time interval related to the time of stimulus presentation, whereas the background EEG activity and non-neural noise occur in a more random fashion. The stimulus and response property means that repetitive stimulation can be used in combination with ensemble averaging techniques
to reduce the noise level [2, 3].

![M-channel single trial evoked potentials in response to stimulus c.](image)

**Fig. 3.3.** M-channel single trial evoked potentials in response to stimulus c.

With a sufficiently low noise level, the time delay (*latency*) and amplitude of each constituent wave of the EP can be accurately estimated and interpreted in suitable clinical terms. The duration, amplitude, and morphology differ considerably from potential to potential as shown in Fig. 3.4.

![Various morphologies of evoked potentials.](image)

**Fig. 3.4.** Various morphologies of evoked potentials.

The use of ensemble averaging is, however, not without complications, since the evoked response, in certain situations, undergoes dynamic changes, thereby violating the averaging assumption of a response exhibiting fixed waveform morphology. One such situation occurs during neurosurgical procedures in which it is important to detect time-varying EP changes related to neurological injury.
Considerable research has been directed toward finding techniques which can track dynamic changes, while at the same time providing sufficient noise reduction. One popular approach is to introduce certain prior information on the behavior of EP morphology, for example, by assuming that each response can be modeled as a linear combination of a subset of orthogonal basis functions. A noise-reduced response is obtained by "reconstructing" the response from a small number of basis functions; the weights of the linear combination result from fitting the basis functions to the observed response. The analysis of time-varying EP changes is commonly referred to as single-trial analysis.

The peak/trough wave components of the EP are referred by the letters P (positive amplitude) and N (negative amplitude). A number is appended to the letter reflecting the latency in milliseconds from the time at which the stimulus was elicited. Alternatively, the appended number may reflect the temporal order of the component, and is then less than ten. For example, P300 signifies that a positive peak occurred at 300 ms, whereas N3 implies that the third waveform component has negative amplitude. It should be noted that EPs, by odd convention, are usually plotted with reversed polarity so that P300 actually corresponds to a trough, and vice versa.

Evoked potentials resulting from auditory (AEP), visual (VEP), and somatosensory (SEP) stimulation are the most commonly used modalities in clinical routine. For all modalities, measurements on latency and amplitude are extracted from the waves of the averaged EP and are compared to normative values in order to discriminate normal, healthy subjects from subjects with various kinds of neurological impairment [55]. Normal values are strongly dependent on age. Therefore, different values have been determined for newborns and adults.

Evoked potentials which have increased latency, decreased amplitude, or
missing are interpreted as abnormal. Evoked potentials are often analyzed in individual channels with respect to temporal and amplitude waveform properties without involving information recorded in other channels. Additional information can be derived on the spatial distribution of voltages on the scalp by simultaneously analyzing all the data in a multichannel recording.

3.5 EVOKED POTENTIAL MODALITIES

A stimulus elicits electrical impulses in local sensory nerve cells which propagate along the nerve fibers to the brain. The sum of all resulting impulses, in combination with the ongoing electrical activity of the brain, constitutes the stimulus response. The elicited impulse is initially spike-shaped with a very short duration, but is prolonged by various factors when recorded by the surface electrode on the scalp. The change in waveform morphology is partly caused by impulse propagation in several parallel nerve fibers with slightly different conduction velocities. Another determining factor is that the surface electrode "views" the neural activity over a rather large region, and, therefore, the morphology of the EP is smoothed. The resulting response reflects impulse propagation from the entrance of the brainstem into various parts of the cortex, shown in Fig.3.5.

However, the amplitude of the brainstem response is smaller than that of the cortex since it originates from a more distant part of the brain with respect to the electrode. During identical stimulus and recording conditions, the brainstem response is about one tenth the size of the cortical response.

Another general property of an EP is that the waves exhibit a gradual slowdown as the response propagates toward the more complex structures of the cortex, i.e., the inter-peak latencies are prolonged with time. Therefore, inter peak latencies of the brainstem response are on the order of a few milliseconds, while late
cortical responses have an inter-peak latency of more than 100 ms.

Fig. 3.5 The cerebral cortex and the four lobes.

3.5.1 Auditory Evoked Potentials

Auditory EPs are generated in response to an auditory stimulation usually produced by a short sound wave. This type of evoked response reflects how neural information propagates from the acoustic nerve in the ear to the cortex. The response can be divided into three different intervals according to latency: the brainstem response, constituting the earliest part, followed by the middle and late cortical responses. Brainstem auditory evoked potentials (BAEPs) have primarily been used for the evaluation of different types of hearing loss ("audiometry"), diagnosis of
certain brainstem disorders, and intra operative monitoring in order to prevent neurological damage during surgery [56].

The waveform characteristics of the middle latency AEP are useful for monitoring the depth of anesthesia during surgery [57, 58]. Since a change in concentration of the anesthetic dose has been found to be closely related to latency, appropriate anesthetic depth can be maintained by continuously tracking changes in latency.

**Recording setup:** Auditory EPs are elicited by a short duration click sound delivered to the subject through a conventional set of stereo headphones. One ear is stimulated at a time, while the other ear is masked with band limited noise ("pink noise"). The click sound is usually produced by a 0.1 ms square wave pulse, having a repetition rate of 8-10 clicks per second. The stimulus intensity is commonly defined in units of peak equivalent sound pressure level and can vary between 40 and 120 dB [59]. Auditory EPs are usually recorded by placing electrodes behind the left and right ear and at the vertex. The placement is identical to that used in EEG recordings.

**Waveform characteristics:** The three parts of the AEP exhibit considerable differences in signal properties. Fig. 3.6 shows (a) Recording setup and (b) a typical brainstem auditory evoked potential. The BAEP has very low amplitude, ranging
from 0.1 to 0.5 µV, and occurs from 2 to 12 ms after stimulus. Due to its low amplitude, several thousands of stimuli are required to achieve an acceptable noise level by averaging. The short duration of the BAEP implies that most of its spectral content is contained in the interval from 500 Hz to about 1.5 kHz [60, 61]. In a normal subject, the BAEP consists of up to seven waves, generated by various neural structures in the auditory pathways. By convention, these waves are labeled with Roman numbers, see Figure 3.6. The loss or reduction of individual waves provides clinically important information, as do absolute and inter-peak latencies. The middle AEP occurs from 12 to 50 ms, and is followed by the late response [56]. The amplitudes of these later components are considerably larger (1-20 µV) than those of the BAEP and increase with latency. One hundred to 1000 stimuli are usually sufficient for adequate noise reduction. While the early brainstem response is quite reproducible from stimulus to stimulus, the middle and late responses can exhibit considerable variability in morphology.

3.5.2 Somatosensory Evoked Potentials

Somatosensory evoked potentials (SEPs) are elicited by electrical stimulation from the body surface of a particular peripheral nerve, usually from an arm or a leg, as shown in Fig. 3.7 (a). This type of stimulation provides valuable information about nerve conduction functionality between the selected stimulation point via the spinal cord to the cerebral cortex. Somatosensory EPs can be used to identify blocked or impaired conduction in the sensory pathways, produced by certain neurological disorders such as multiple sclerosis [12]. Another application of the SEP is intraoperative monitoring during spine surgery; unchanged waveform morphology throughout surgery suggests that no deterioration in neurological function has taken place.
**Recording setup:** Stimulation is performed by delivering a brief electrical impulse via two stimulus electrodes positioned close to the sensory nerve fiber. Similar to the recording of AEPs and VEPs, SEPs are recorded by placing electrodes over the motor sensory cortex at predefined locations. However, a number of additional electrodes are needed, and these are positioned along the nerve pathway to the cortex, e.g., on the knee and the spinal cord. In clinical routine, SEPs are usually recorded by stimulation of three different nerves: the median nerve in the arm and the tibial and peroneal nerves which are both in the leg.

Fig.3.7. Somatosensory evoked potentials. (a) Recording setup and (b) a typical somatosensory evoked potential when the peroneal nerve of the leg is stimulated.

**Waveform characteristics:** The SEP has most of its spectral content in an interval located well above 100 Hz. Fig.3.7 (b) shows a typical waveform of somatosensory evoked potentials. The total SEP duration is about 400 ms; however, only the first 40 ms are commonly recorded and analyzed because the long-latency response exhibits large variability. Similar to the AEP, the SEP amplitude has substantial inter subject variability and, therefore, is of limited clinical value. Important diagnostic information derived from the SEP waveform is
conveyed by the absence of peaks, slow conduction velocities, and electrode-to-electrode variations in conduction velocity.

### 3.5.3 Visual Evoked Potentials

The electrical response elicited by visual stimuli can be recorded from the occipital region of the scalp for the evaluation of visual pathway functionality. Two different types of stimulus are used, pattern reversal and flashing, depending on the suspected disorder and the ability of the subject to cooperate during the recording procedure as shown in Fig. 3.8. The clinically useful information from visual evoked potential (VEP) is extracted from the later parts of the response, starting at about 75 ms, and, accordingly, the VEP is referred to as a long-latency response [62]. Visual EPs are used for investigating ocular and retinal disorders and for detecting visual field defects and optic nerve pathology. It has also been suggested that the VEP be used for intra operative monitoring where the aim is to detect early changes in waveform morphology in order to avoid visual loss and damage to the optic nerve.

![Recording setup](image)

**Fig. 3.8.** Visual evoked potentials. (a) Recording setup where pattern reversal is used as the stimulation and (b) typical visual evoked potential morphology.

**Recording setup:** The recording of a VEP is often based on a pattern reversal stimulus, generated by a chessboard pattern being displayed on a video screen as shown in Fig.3.8 (a). During the investigation, the patient is required to focus on a
point in the center of the screen while the black-and-white squares are reversed at a fixed repetition rate so that the white squares become black, and vice versa. Typically, a rate of two reversals per second is used. The size of the chessboard squares, the luminance and contrast of the squares, and the repetition rate exemplify factors which influence the waveform amplitude and latency. These factors must therefore be taken into account when interpreting the VEP; the factors can obviously be manipulated in order to infer additional information from the VEP. The use of flash stimulus is considered when the patient is unable to either focus or maintain the level of fixation required for pattern reversal stimulation. For example, flash stimulation can be helpful when suspected vision disorders are investigated in neonates. Flash stimulus is delivered at a rate of five to seven flashes per second. Although the eyes are closed during this procedure, a sufficient amount of light will pass through the eyelids to activate the retina. The recording electrodes are positioned at locations close to the visual cortex, and the reference electrode is placed at the vertex.

**Waveform characteristics:** Fig.3.8 (b) shows typical morphology of visual evoked potentials. The VEP has an amplitude which is considerably larger than that of an AEP or SEP, ranging up to 20 µV. As a consequence, the VEP is the only type of EP that, at best, can be observed directly in the EEG without prior noise reduction. However, such reduction is performed in clinical routine in order to assure a sufficiently low noise level; typically 100 stimuli are needed to achieve that level. The spectral components of a VEP range, in rough terms, from 1 to 300 Hz. The P 100 wave can occasionally exhibit a split ("bifid") morphology, which may be indicative of abnormality. From a signals analyst's point of view, the presence of a bifid morphology implies that the high-frequency content of a VEP increases. In a normal subject, the VEP waveform configuration is described by a small positive peak, a
larger negative peak occurring about 75 ms after stimulus (N75), and a large positive peak about 100 ms after stimulus (P100). The duration of the response may extend beyond 300 ms. The absolute latency of P100 as well as differences in P100 latency between the left and right eye are important measurements which are useful for diagnosis [1].

**Visual oddball Paradigm:** Visual event-related potentials (VEPs) were obtained with a checkerboard pattern (side length of checks: 50' visual angle). A sequence with two different stimuli was presented pseudo randomly: the frequent or non-target stimuli were a colour reversal of checks (80% of the stimuli), while the less frequent or target stimuli were colour reversals with a half check displacement (both horizontal and vertical) of the pattern (20% of the stimuli). Subjects were asked to ignore the non-target stimuli and press a key whenever they saw the target ones. Each pattern reversal was shown for 1 sec and the inter stimulus interval varied pseudorandomly between 2 and 2.2 sec. No two target stimuli appeared in succession. Subjects were asked to fixate on a small red circle in the centre of the screen during the recording.

### 3.6 EVOKED POTENTIALS AND COGNITION

The above three EP modalities represent different types of response to physical stimuli and are therefore referred to as "exogenous" responses. However, EPs can also be elicited by various cognitive factors ("endogenous" responses), resulting in a late response with latencies of 300 ms and longer [10, 63]. The most well-known, late-latency peak is P300 which is considered to reflect the cognitive capability of a subject, involving higher mental functions such as attention and memory processes. The P300 latency is related to the time required for memory updating associated with a given task; in general, latency decreases for increased cognitive performance [64].
The P300 is commonly elicited by means of an "oddball" task in which two different stimuli, for example, two tones with different pitch, are presented at random to the subject, although one of the stimuli occurs more infrequently. The task of the subject is to indicate, by pressing a button, when the infrequent stimulus occurs (i.e., the oddball) but not when the frequent one occurs. The responses related to the infrequent stimulus are then averaged, and the resulting waveform is analyzed [65]. Evoked potentials have also been considered in the study of language comprehension, where it is of interest to understand how the normal brain constructs meaning from words in real time. It has been shown that a negative peak at around 400 ms (N400) varies systematically when semantic information is being processed. For example, it has been observed that a stimulus defined by a semantically anomalous word in a sentence context produces an electrical response. The amplitude of the N400 in response to such an "outlier" word is sensitive to the local context in which it occurs; words which are in context are easier to process for the brain and, therefore, elicit smaller N400 amplitudes than do words which are out of context. The amplitude of the N400 peak has also been found to be sensitive to the ease with which information is accessed from long-term memory.

Fig. 3.9. Visual evoked potentials of normal and abnormal subjects.
3.7 NOISE CHARACTERISTICS

In the analysis of evoked potentials, noise is essentially the spontaneous background EEG activity. Thus, the EEG is the target activity when methods for noise reduction are discussed below. Non-cerebral noise sources, hampering the success of EEG signal processing, must also be taken into account when EPs are processed. The main non-cerebral sources are eye blinks, eye and eyelid movements, muscle activity, 50/60 Hz power line interference, instrumentation noise, and poor electrode attachment. Linear, time-invariant, band pass filtering is sometimes used to remove noise whose spectral content is outside that of the EP.

Certain types of noise and artifacts are related in time to the stimulus and, therefore, will degrade the performance of ensemble averaging methods. Eye movement represents the most important source of such artifacts and distorts the signal to various degrees depending on the direction of the eye movement and the electrode position. The influence of such ocular activity can be substantially reduced by computing an ensemble average of visual evoked potentials.

The electrical activity of the heart is another noise source whose contribution is difficult to cancel with ensemble averaging techniques, especially when the heart rate happens to coincide with the stimulus rate. Information on heartbeat timing can, however, be acquired so that a stimulus rate can be selected which precludes this type of time-locked artifact [36]. Another technique for avoiding such artifacts is to use an aperiodic presentation of the stimulus [24, 66].

3.8 NOISE REDUCTION BY ENSEMBLE AVERAGING

Averaging techniques are used to extract the signal related to the stimulus and reduce the amplitude of the ongoing EEG signal (noise) leading to an improvement in the signal-to-noise ratio (SNR) of the measured response (noisy EP). The averaging
operation is performed prior to analysis and classification. Therefore, the results obtained and the conclusions drawn about the EP are highly dictated by the accuracy of the averaging operation.

Sometimes potentials are also emitted without any clear physical stimulus. Therefore it is currently common to use the notion of event related potentials (ERP). The evoked potentials are then event related potentials that are immediate or intermediate responses to physical stimuli. Examples of such stimuli are electric stimuli, visual stimuli, and auditory stimuli.

The choice of stimulus type to be used depends on the part of the nervous system to be investigated and the circumstances under which measurements are to be made. A visual evoked potential (VEP) is generated by presenting a visual stimulus to a subject. Stimuli that may be presented include alternating check board patterns, light flashes and appearing and disappearing diffuse light patterns. VEPs are usually presented by means of a television screen or light emitting diodes (LEDs).

The pattern of the VEPs is affected by elements of such as the subject’s age and sex, the type of stimulation, the contrast and intensity of the stimuli, and location of the recording electrodes. Most often, transient check board VEPs are used to study the conduction in the visual pathway.

A somatosensory evoked potential (SEP) is usually generated by the application of an electrical current to the arm or leg using surface electrodes or needle electrodes. The signal is passed along the peripheral nerves and the spinal cord, to the sensory cortex. Many factors such as location of the stimulus electrodes, the amplitude and repetition rate of stimuli, and the length of the limbs and the age of the subject can influence the SEP. Somatosensory evoked potentials are often used to detect and localize lesions and potential damage in the somatosensory pathway.
Auditory evoked potentials are generated by delivering clicks or tone bursts. This is done via ear phones. AEPs are measured using scalp electrodes on the vertex and ear lobes of the patient. Auditory evoked potentials typically contain a sequence of positive and negative peaks or waves. These peaks are generated in specific parts of the neural pathway. In audiologist studies AEPs can be used to detect and assess hearing defects in non communicative subjects such as small children and mentally disabled people. AEPs are used in monitoring depth of Anesthesia.

Evoked potentials are used in various clinical and laboratory applications. They are used to test conduction in the visual, auditory, and somatosensory systems. During surgery they can be used to monitor the condition of structures at the operative site. Sensory evoked potentials can also be used for monitoring effects of anesthetics on the central nervous system (CNS).

Evoked potentials (EPs) are brain responses that are time-locked to the onset of an external event. Using surface electrodes a sequence of positive and negative peaks can be recorded; such a sequence is called a sensory evoked potential.

These peaks are characterized by their amplitude and time after the stimulus at which they occur as the (post stimulus) latency. Artifacts are defined as patterns in the training set that lead to inaccurate estimation of classifier parameters and patterns in the test set that yield misleading performance evaluations. Artifacts can give inaccurate test results which can have serious consequences, for example, inaccurate diagnosis in clinical evaluations.

Although efforts are made to carefully collect the patterns in practice, the ensembles are often contaminated with artifacts. It is clearly more prudent to repeat a test if an artifact can be detected rather than to have an inaccurate test result. Artifacts in EP/EEG brain waveform recordings typically result from voltage changes due to
eye blinks, eye movements, muscle activities, and power line noise.

3.9 ARTIFACTS

Assuming that the majority of single trial evoked potential responses are normal, an artifact is defined as a single trial that is not similar to the underlying evoked potential of that ensemble. Artifacts in EP/EEG brain waveform recordings typically result from voltage changes due to eye blinks, eye movements, muscle activities, and power line noise.

If a signal contains few samples with more deviation from mean when compared to normal ones, then it is said to be type-A artifact. A signal containing majority of samples with slightly increased amplitude levels is said to be type-B artifact. Either of these artifacts cannot be identified visually, but affects statistical parameters.

3.10 NEED FOR EVOKED POTENTIALS

Diagnosis of diseases in human body requires the knowledge of pathological changes that occur with time. Brain reacts in different manners for different types of stimuli applied. It receives signals from the sensory organs eyes, skin, ears, etc., and reacts in different manners for different signals. If the brain is not able to discriminate the differences in the signal received from any one of these sensory organs, even though they sensory organs are in good condition, then we say that there are some problems in the neural pathways connecting sensory organs to the brain. In such situations evoked potentials are needed to detect the neurological problems. For example the latency and amplitude levels of positive and negative peaks in visual evoked potentials are used to detect the abnormalities in visual pathways.
3.11 CONCLUSIONS

This chapter covered biomedical signals, ECG, EEG and evoked potentials; types of evoked potentials, visual evoked potentials, auditory evoked potentials and somatosensory evoked potentials. Artifacts and types of artifacts are defined. Need for evoked potentials and state of research in this area is also discussed.