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Amygdala Neuronal Responses to Prepulse Inhibition during Rat Startle Behavior.

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Abstract

Prepulse inhibition, a phenomenon in which a weak pre-stimulus suppresses the startle response to an intense noise stimulus, is disrupted by lesions of limbic cortical structures including amygdala. In the present study, neuronal activity in rat amygdala was recorded to clarify contributions of the amygdala in the prepulse inhibition of a startle behavior. The head of each animal was fixed chronically in a stereotaxic frame in a painless state, and neuronal activity of amygdala was stereotaxically recorded in the behaving animal. Startle response in behavior was suppressed to prepulse trials even in the head-fixing awake condition as well as in the free moving condition. Of 335 amygdalar neurons recorded, 100 (29.9%) responded during such a startle behavior. Of these, 76 (22.7%) responded at all of noise-alone trials and prepulse trials of 1 and 12 dB above the background noise (excitation, 49; inhibition, 27). Twenty-four neurons (excitation, 20; inhibition, 4) responded differentially during prepulse trials. These suggest that the amygdala involves neuronal control of prepulse inhibition as well as startle behavior.

1. Introduction

The acoustic startle reflex is a short-latency response involving a rapid muscle extensions/flexions elicited by a sudden and intense auditory stimulus [9, 10]. The amplitude of the startle reflex is reduced when the strong startle-eliciting stimulus is preceded shortly by a weaker stimulus, or prepulse [8]. This is known as prepulse inhibition (PPI), which might be related to sensorimotor gating or filtering of the sensory information [2]. PPI in schizophrenic patients is deficient [3]. Several papers suggest that limbic cortical structures contribute to modulation of sensorimotor gating [9]. The amygdala, a major structure in

the limbic system, receives various cortical and subcortical sensory inputs[14, 21]. The lesion study showed that the amygdala plays an important role of control of startle plasticity[5,7], and also contributes to control of the PPI throughout the dopamine circuits[4, 23]. In the present study, neuronal activity in rat amygdala was recorded to clarify contribution of the amygdala in the prepulse inhibition of a startle behavior.

2. Materials and Methods

Subjects Seventeen male albino Wistar rats, weighing 300-350g, were used. Each animal was individually housed in clear plastic cages. Animals were allowed access to food and water ad libitum throughout the experiment.

Surgery Nine rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and restrained in a stereotaxic apparatus. The skull exposed, and six small holes were fastened to the skull as anchors. One of small screws was used as ground reference. A receptacle for four modified ear bars was then formed of dental cement built up around the working region to permit the animal's skull to be later fixed painlessly in the correct stereotaxic pl anes. After completing the surgery, the wound was cleaned, and the scalp was sutured. The rats were returned to their home cage after surgery, and allowed 10-14 days for recovery. On the day before beginning unit recording, a 2-mm-dam hole was drilled, under ketamine anesthesia (30 mg/kg, i.m.), in the skull over the intended recording site (A1.8-3.6 mm, L3.0-5.5 mm from bregma), and covered with a thin dental cement. The animals were again returned to their home cages. Just before unit recording, the dura mater was incised with a fine needle for electrode insertion under local anesthesia by a drop of 1% lidocaine.

Equipment The startle chamber (SR-LAB, San Diego Instruments, San Diego, CA) was used in a sound-insulated box. The box consisted of a clear nonrestrictive cylinder resting on a platform inside of a ventilated and illuminated chamber. A high-frequency loudspeaker inside the chamber produced both a continuous background noise of 60 dB and the various acoustic stimuli. Magnitude of the body startle responses was detected by a piezoelectric accelerometor mount below the Plexiglas frame. The signals were digitized and stored by an interface unit and a microcomputer.

Behavioral procedure The rat was placed in the startle chamber for 5 min with a background noise level of 60 dB. In the testing session, animal was exposed to six types of trial: (1) a pulse trial in which a 40 msec 120 dB acoustic noise burst alone was presented, (2) four prepulse plus plus trials in which 20 msec noise that were either 1, 3, 6, 12 dB

above the background noise were presented 80 msec before the onset of the 120 dB pulse, and (3) a trial which included only background noise. All types of trial were presented 10 times in a pseudorandom order, with a intertrial interval of 10-20 sec.

Fixed session Head of the rat was fixed in a special stereotaxic apparatus. The startle chamber was modified for a stereotaxic situation. The loudspeaker was removed from the chamber and was set on the stereotaxic frame at the position of 25 cm hight. The cylinder chamber with an accelerometer was set between stereotaxic parallel frames. All trials were controlled by SR-LAB system.

Unit recording session The rat was fixed on a similar condition as the previous fixed session. In this session, pulse-alone, 1 and 12 dB PPI trials with each 5 trial were carried out.

Recording procedures and data analysis An extracellular action potential was recorded with bipolar glass-coated tungsten electrode extending 30-50 μm beyond the tip. Signals of unit activity were amplified with a conventional preamplifier and converted to standardized pulses in a time window discriminator. The signal was then transferred to a conventional computer to make a pre-post stimulus histogram. Also the signals were recorded in the tape recorder for the later off-line analysis.

Histology Following the conclusion of the experiment, the rats were given an overdose

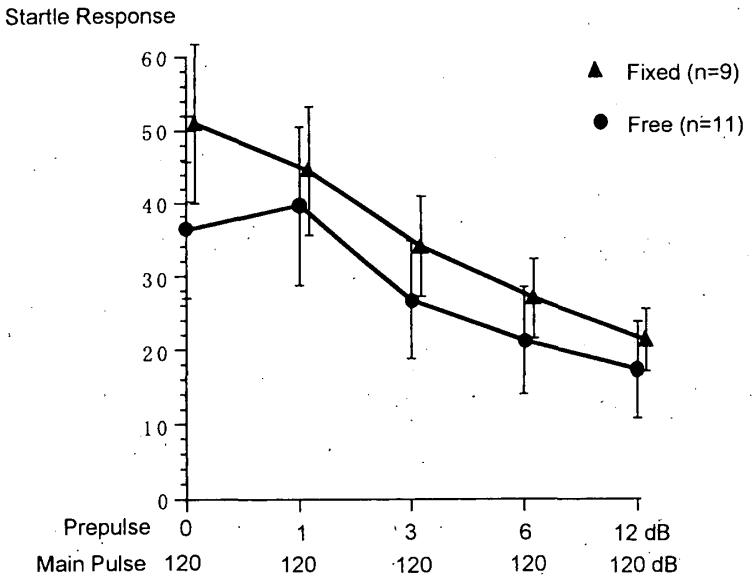

Fig. 1. Magnitude of startle responses during the PPI tests in the testing chamber (●, n=8) and in the head-fixed condition (▲, n=9). Vertical axis, averaged startle responses (average over 500 msec). Horizontal axis, amplitude of prepulse noise to 0, 1, 3, 6 and 12 dB above the background noise of 60 dB. The startle response decreased with amplitude of prepulse both during free moving condition and during the head-fixed condition.

of sodium pentobarbital, and perfused with 10% buffered formaline solution. The brain was removed and soaked in 10% buffered formaline. Fifty μm thick sections were cut and stained with cresyl violet. The extent of the lesion was assessed microscopically.

3. Results

Startle behaviors in the head-fixing condition was at first compared with those in the testing chamber to confirm reapppearance of the PPI in the different situation. Figure 1 shows magnitude of startle responses during the PPI tests in the testing chamber and in the head-fixed condition. The startle amplitude was suppressed by the 1, 3, 6 and12 dB of prepulse trials in the freely moving condition. The amplitude of the startle stimuli in the head-fixing condition was again decreased with the prepulse stimulation although the

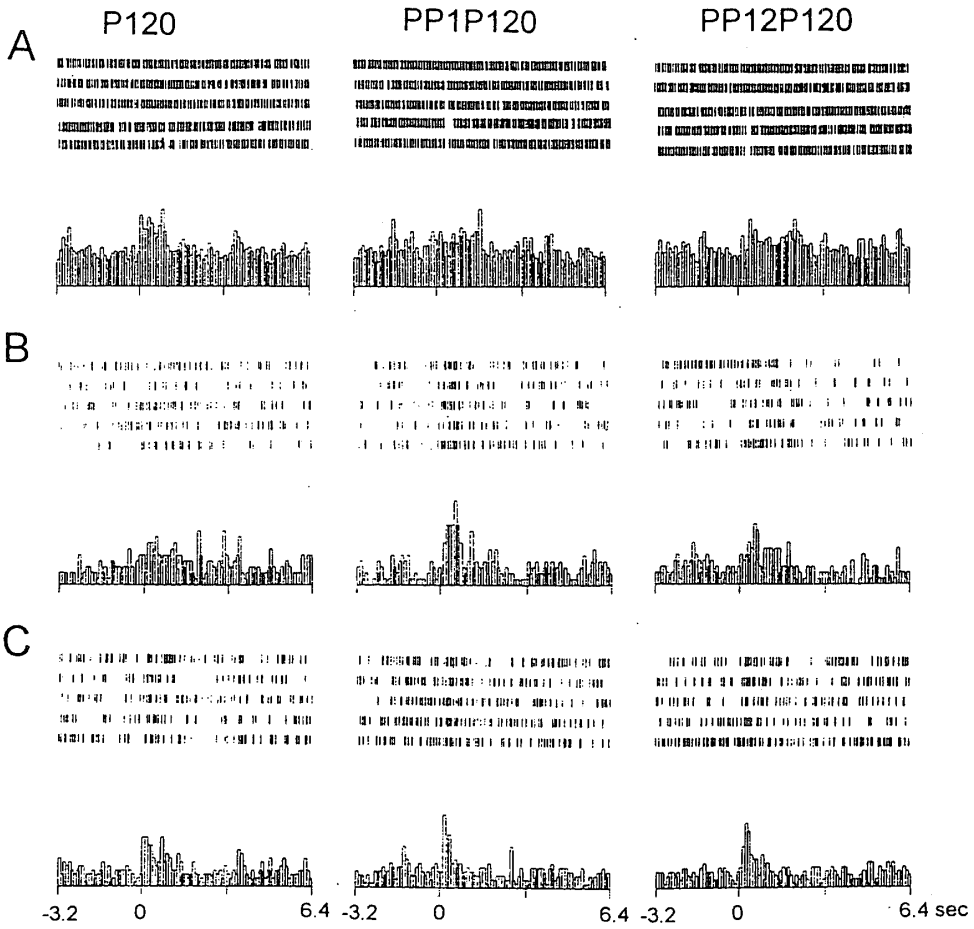

Fig. 21. Examples of neuronal activities in the three different amygdala neurons during the PPI tests. P120, trial with only 120 dB noise stimulus. P1P120, P12P120, trials with 1 dB or 12dB above the background noise as prepulse amplitude. A and B. Examples of neurons responded differentially to noise-alone, or prepulse trials. C. Examples of neurons responded non-differentially to noise-alone and prepulse trials.

amplitude is slightly higher as shown in the Fig. 1.

Of 335 amygdalar neurons recorded, 100 (29.9%) responded during noise-alone and/or prepulse trials (Table 1). Of those, 24 (7.2%) responded differentially to the noise-alone or prepulse trials. Figure 2 shows examples of neuronal activities in the three different amygdalar neurons during the PPI tests. Some neurons responded differentially to noise-alone (Fig. 2A), or prepulse trials (Fig. 2B). Another neurons responded non-differentially to noise-alone and prepulse trials (Fig. 2C). Of differential responded neurons, 11 responded only prepulse trials (Table 1).

Table 1. Summary of the responding and recording neurons.

Recorded Neurons 335
Responded neurons 100 (29.9%)
Non-differential responses 76 (22.7%) (↑,49; ↓,27)
Differential responses 24 (7.2%)

P120	P1P120	P12P120	
↑	—	—	6
↓	—	—	1
—	↑	—	3
—	↑	↑	7
—	↓	↓	1
↑	↑	—	4
↓	↓	—	1
↓	—	↓	1

4. Discussions

Our experimental data show that the some amygdala neurons involve in the regulation of the PPI. The amplitude of the startle response is reduced when the strong startle-eliciting stimulus is preceded shortly by a weaker stimulus. This is thought to reflect the process of sensorimotor gating under the influence of mesoaccumbal dopamine system[18,20]. The PPI is disrupted by excessive stimulation of dopamine receptors on medium spiny neurons of the ventral striatum[19]. The amygdala connects with the ventral striatum. Basolateral amygdala (BLA) electrical stimulation induces either excitation or inhibition of ventral striatum neurons. Quinolinic acid lesions of the BLA significantly reduced PPI without significantly changing startle amplitude[23]. These suggest that the amygdala plays a role in sensorimotor gating mechanisms.

Anatomically, the amygdala receives information from all sensory modalities via the association cortex and from the thalamus[11]. Auditory information comes from auditory cortex and thalamus[12]. Amygdalar neurons responded to the auditory stimulus associated

with conditioned reward and aversion [13, 22]. In the fear conditioned task associated with tone, the neuron in lateral amygdala responded to the conditioned stimulus [1, 15~17]. This suggests that the amygdala neurons code auditory information in learning. Our data supported this hypothesis. However, it is unclear where the origin to startle tone information is in the startle reflex. The tone signal goes initially to cochlear nucleus through VIII nerve root, and then to the ventral half of the nucleus reticularis pontis caudalis (RPC) [6, 24]. The RPC neurons projects directly to the motoneuron in the spinal cord that induces the body reflex. On the other hand, the tone signal projects to the thalamus through the cochlear nucleus, and to the lateral nucleus of the amygdala [11]. This projection of the tone information might induce the tone responsiveness in the amygdala.

We observed selective neurons that responded only during the PPI trials. It is reported that the PPI might regulate in the mesolimbic system in the CNS. Some feedback information might send to the amygdala from the accumbens nucleus, for example.

Acknowledgments

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