Enhancement of electrically evoked startle-like responses by tetanic stimulation of the superior colliculus

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Single-pulse unilateral electrical stimulation of either the amygdala or the inferior colliculus elicited startle-like responses in chloral hydrate anesthetized rats. EMG responses to intracranial stimulation were recorded from the anterior biceps femoris muscles. The EMG responses were generally enhanced following unilateral tetanic stimulation of the deep layers of the superior colliculus, but the enhancement was stronger for amygdala sites than inferior colliculus sites. The enhancement of EMG responses to ipsilateral amygdala stimulation was much larger than that for contralateral amygdala stimulation and that for ipsilateral inferior colliculus stimulation. The enhancement of EMG responses to contralateral inferior colliculus stimulation was not significant. The present study provides a motor-output model for studying plasticity in the neural pathways mediating startle facilitation. *NeuroReport* 13:1769–1773 © 2002 Lippincott Williams & Wilkins.

Key words: Amygdala; Deep layers of the superior colliculus; Deep mesencephalic gray; Electrical stimulation; EMG; Fear potentiation; Inferior colliculus; Mesencephalic reticular formation; Plasticity; Startle reflex; Summation

INTRODUCTION

The startle reflex is the most extensive of all reflexes across mammalian species, involving a rapid contraction of skeletal muscles along the full length of the body following a sudden and intense sensory stimulus. One of the most striking features of startle is summation across tactile, auditory and vestibular modalities [1].

The amygdala plays an important role in fear potentiation of startle [2,3]. Electrical stimulation of the amygdala can elicit startle-like responses [4–6], while electrical or chemical activation of the inferior colliculus (IC) can result in both defensive responses [7] and startle-like responses [8]. Previous studies, however, have not investigated summation and functional coordination between amygdala and IC outputs in regulating aversive emotion and startle.

Both lesion and electrical stimulation studies have suggested that strong synaptic relays in the rostrolateral midbrain mediate both expression of fear-potentiated startle and startle-like responses evoked by electrical stimulation of the amygdala [4,5,9,10]. This midbrain area includes the deep layers of the superior colliculus (DpSC), the deep mesencephalic reticular formation, and the deep mesencephalic gray. In rats, electrical stimulation of this area can evoke startle-like responses [4] and fear-like behaviors [11–13].

In rats, the DpSC is one of the major targets receiving axonal projections from the external nucleus of the IC [14] and sends direct projections to the caudal pontine reticular nucleus [9], which is the critical premotor structure mediating the acoustic startle reflex (for recent reviews see [1,15,16]). Therefore, as indicated in Fig. 1, the DpSC and its subjacent areas can be a relay and/or integration site mediating amygdala and IC startle-inducing stimuli.

Long-lasting changes of synaptic strength, such as long-term potentiation (LTP), can result from stimulation both forebrain and brainstem structures. Tetanic electrical stimulation can induce LTP in *in vitro* preparations of the amygdala, superficial layers of the SC, and the IC [17–19].

The goal of the present study was to investigate whether startle-like responses to electrical stimulation of the amygdala or IC can be used as a model for studying plasticity in the pathways regulating aversive emotion and startle. Electrically evoked startle-like EMG responses were examined before and after tetanic stimulation of the DpSC.

MATERIALS AND METHODS

Subjects were 25 male adult Wistar rats (*Rattus norvegicus*; 175–350 g), obtained from the China Academy of Military

Medical Sciences (Beijing, China). The rats were anesthetized deeply with 10% chloral hydrate (400 mg/kg, i.p.) and placed in a Kopf stereotaxic head holder. A state of areflexia was maintained throughout the experiment by supplemental injection of the same anesthetic. Flexible wire electrodes were implanted into the hindlimb anterior biceps femoris muscles for measuring EMG responses. A midline incision was made in the head scalp, and the skin and temporal muscles were retracted laterally. The animal head was positioned with bregma and lambda in the same horizontal plane. Craniotomies were made on the dorsal surface of the skull to permit insertion of stimulation electrodes into the brain.

Stainless steel electrodes [20] were aimed at the following three brain structures referenced to bregma, and based on the coordinates provided by [21]: (i) central nucleus of the amygdala (Ce): AP = −2.8 mm, ML = 4.5 mm, DV = −8.0 mm; (ii) IC: AP = −8.8 mm, ML = 1.5 mm, DV = −4.5 mm; (iii) DpSC: AP = −5.8 mm, ML = 1.3 mm, DV = −5.2 mm.

Electrical stimuli and recordings: Electrical stimuli were generated by a Grass S-88F stimulator (Grass, Quincy, Massachusetts, USA), which provided monophasic cathodal rectangular pulses (duration 0.2 ms) via constant-current, photoelectric stimulus-isolation units (model PSIU6). Stimuli in the Ce and IC were single pulses; tetanic stimuli in the DpSC were pulse trains (train length 1000 ms; train interval 1000 ms; train number 10; pulse frequency 250 Hz). Hindlimb EMG signals were filtered and amplified by a Cornerstone amplifier (EX4-400), with a band pass between 300 and 1000 Hz. Peak-to-peak amplitudes of the primary EMG responses were displayed and measured on a digital real-time oscilloscope (Tektronix, TDS 220). Figure 1 illustrates the arrangement of electrodes for the tetanic stimulation experiments.

Single-pulse unilateral stimulation of the Ce or the IC was first tested to establish the minimum current (the threshold current) required to elicit clearly detectable startle-like hindlimb EMG responses (e.g. bilateral, short-latency) following each stimulus. Once this threshold current was determined, nine stimulus current levels were used for building the amplitude-current curve, six above the threshold, one on the threshold and two below the threshold. The current interval was 50 μA. EMG responses were measured before and during the 30 min immediately after tetanic stimulation of the DpSC. Six trials were assigned to each current level, and the order of presentation was arranged in a pseudo-random manner. The current of unilateral tetanic stimulation of the DpSC was set 100 μA above the threshold level for single-pulse DpSC stimulation. The inter-trial interval was 30 s. Statistical tests applied to the data were within-subject two-way ANOVA.

At the end of testing, the rats were sacrificed with an overdose of chloral hydrate. Lesion marks were made via the stimulating electrodes by an anodal DC current (500 μA for 10 s). The brains were removed, stored in 10% formalin with 30% sucrose until they sank, and then sectioned at 40 μm in the frontal plane in a cryostat (−20°C). Sections through the stimulation sites were stained with cresyl violet to determine electrode locations.

RESULTS

Histological examinations verified that all the electrode locations were in the correspondent brain regions for the 25 rats used in the present study. The locations of electrode tips aimed at the Ce, IC and DpSC are indicated in Fig. 2. The left central nucleus of the IC was stimulated in 11 rats; the right Ce was stimulated in 14 rats.

Electrically evoked startle-like EMG responses: In chloral hydrate-anesthetized rats, single-pulse unilateral electrical stimulation of the Ce or the IC produced short peak latency (about 8 ms) and bilateral EMG activity recorded from the hindlimb anterior biceps femoris muscles. The hindlimb EMG activity was always accompanied by bilateral whole-body responses that were similar to those elicited by stimulation of the trigeminal, cochlear or vestibular nucleus [22–24]. Thresholds of hindlimb twitches induced by electrical stimulation appeared higher than those for neck and back twitches. The cross-subject average EMG response thresholds for Ce and IC stimulation were 410.9 ± 72.6 μA and 390.0 ± 61.8 μA, respectively. The difference was not significant ($F(1,23) = 0.234$, $p = 0.634$).

Effects of tetanic stimulation of the DpSC: Figure 3 shows the effects of tetanic stimulation of the DpSC on EMG responses elicited by stimulation of the Ce or IC for the four groups of rats with different stimulus combinations: (1) Group A, right Ce/right DpSC (7 rats); (2) Group B, right Ce/left DpSC (7 rats); (3) Group C, left IC/left DpSC (6 rats); (4) Group D, left IC/right DpSC (5 rats). Response amplitudes were normalized relative to the maximum response before tetanic DpSC stimulation. When currents of stimuli delivered to the Ce or IC were increased, the peak startle amplitude progressively augmented for each of the four animal groups. Following tetanic DpSC stimulation, whose mean current was 581.6 μA, the amplitude–current curves for all the animal groups generally shifted upwards compared with the baseline tests before tetanic stimulation of the DpSC.

As shown in Fig. 3a, tetanic stimulation of the right (ipsilateral) DpSC resulted in strong enhancement of EMG responses to stimulation of the right Ce. The largest average enhancement reached a peak of three times the maximum amplitude before tetanic stimulation. ANOVA with repeated measures on the effect of tetanic stimulation revealed a significant effect ($F(1,6) = 13.533$, $p = 0.010$).

Tetanic stimulation of the left (contralateral) DpSC also resulted in a significant enhancement of EMG responses to stimulation of the right Ce (Fig. 3b; ($F(1,6) = 11.009$, $p = 0.016$). The enhancement was smaller than that for stimulation of the right (ipsilateral) DpSC. The largest average EMG amplitude after tetanic stimulation was less than twice the maximum amplitude before tetanic stimulation.

Tetanic stimulation of the DpSC led to less increase of EMG responses to stimulation of the IC, compared to EMG responses to stimulation of the ipsilateral Ce. As shown in Fig. 3c, tetanic stimulation of the left (ipsilateral) DpSC significantly increased EMG responses to stimulation of the left IC ($F(1,5) = 17.459$, $p = 0.009$), but the increase in the peak amplitude was only about 60%.

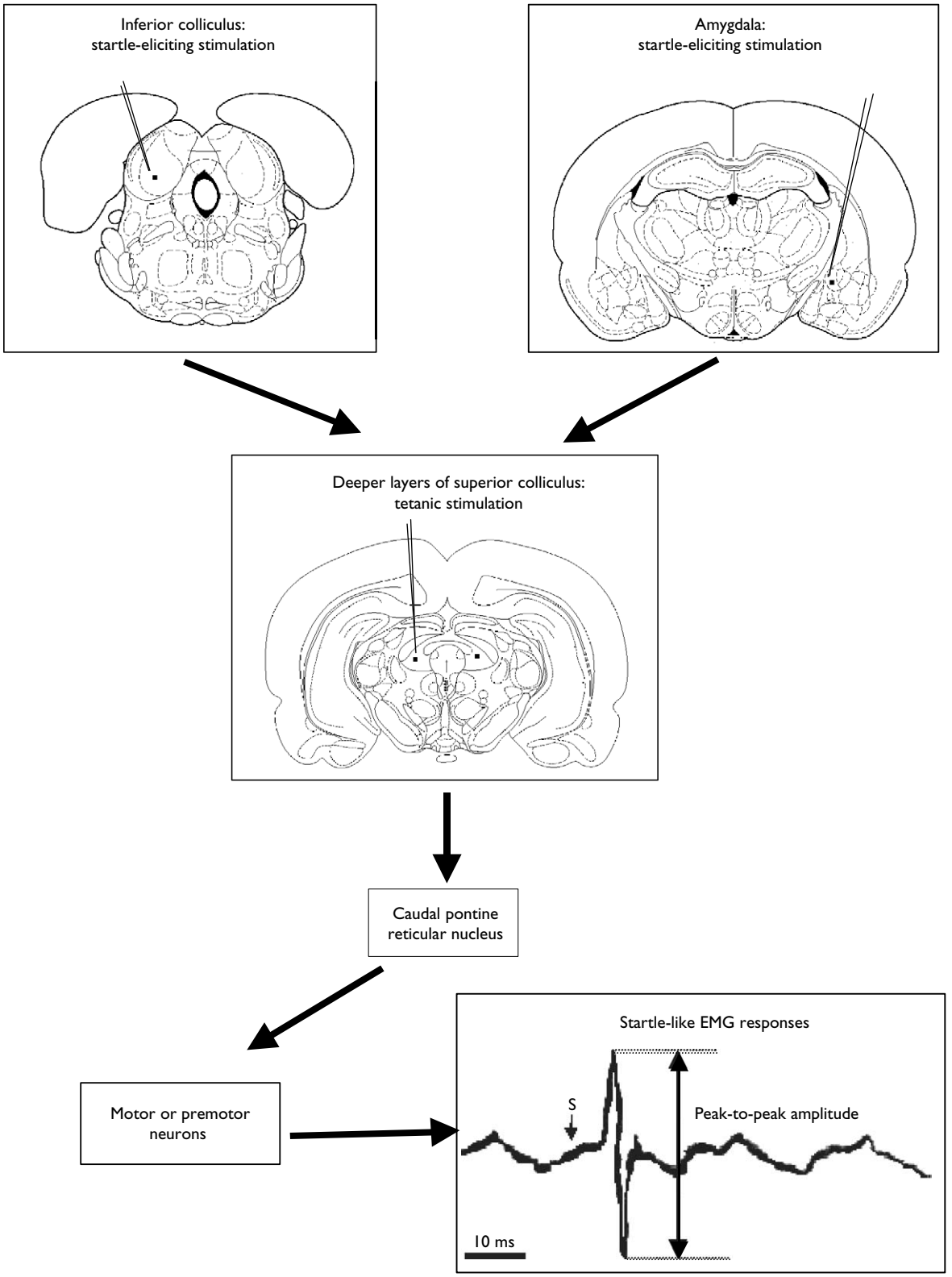

Fig. 1. Schematic diagram illustrating a model of neural circuits mediating fear potentiation of the startle reflex, and showing the methods used in the present study. A copy of EMG waveform from the oscilloscope screen is displayed in the bottom panel, in which the onset of stimulus (S) is indicated by an arrow pointing down and the peak-to-peak amplitude is indicated by a double-headed arrow.

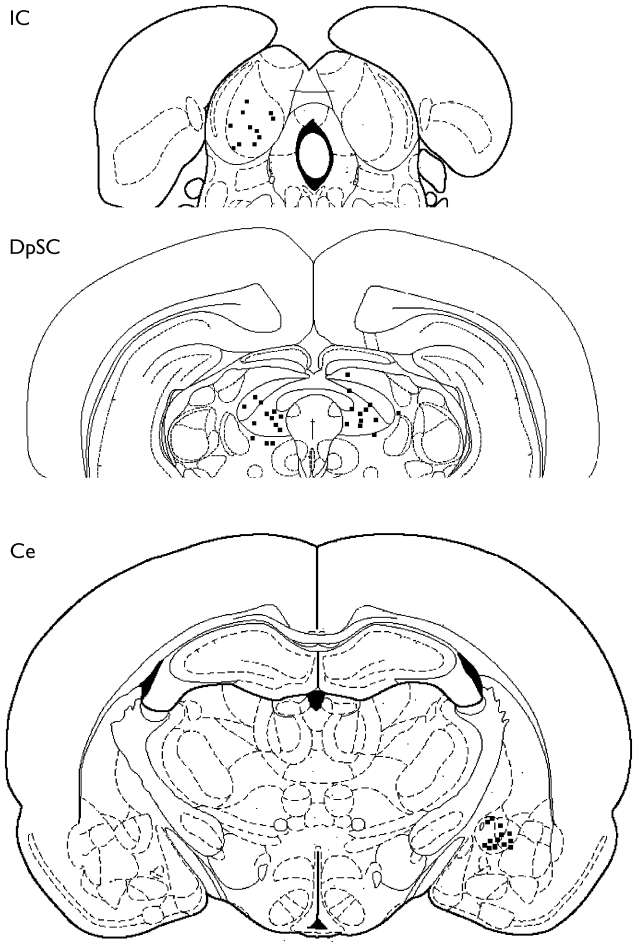

Fig. 2. Locations of electrode tips (squares) in the regions of the inferior colliculus (IC), the deep layers of the superior colliculus (DpSC) and the central nucleus of the amygdala (Ce) for 25 rats. The brain section diagrams were based on [21].

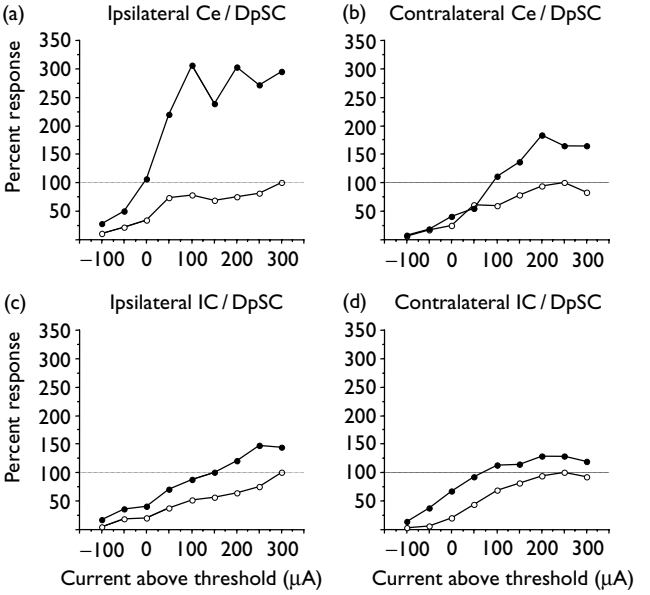

Fig. 3. Normalized amplitudes of the startle-like EMG responses to stimulation of the Ce (**a**,**b**) or the IC (**c**,**d**) at the nine stimulating current levels relative to the threshold determined before unilateral tetanic DpSC stimulation. Open and closed circles represent responses before and after tetanic DpSC stimulation, respectively. Response amplitudes were normalized relative to the maximum response obtained before tetanic DpSC stimulation (the highest open circle).

A slight increase of EMG amplitude following tetanic stimulation of the right (contralateral) DpSC was observed when EMG responses were elicited by stimulation of the left IC (Fig. 2d). ANOVA with repeated measures on the effect of tetanic stimulation revealed that the average EMG amplitude change was not significant ($F(1,4) = 1.267$, $p = 0.323$).

DISCUSSION

The amygdala and the IC are two structures where fear-like and defensive behaviors can be evoked by electrical stimulation [2,3,7]. In the present study, single-pulse unilateral electrical stimulation of the Ce or the IC elicited short latency, whole-body, bilateral, startle-like responses in chloral hydrate anesthetized rats. These results are consistent with the previous reports that electrical stimulation of the amygdala or the IC elicits startle-like responses in awake rats [4,5,6,8]. The amygdala and the IC must have close functional connections with the primary startle pathway. The stimulation currents used for Ce stimulation could have activated nearby internal capsule axons. Activation of the internal capsule axons can evoke non-startle-like unilateral forelimb flexions [25], but may not significantly influence bilateral startle-like responses of the hindlimb anterior biceps femoris muscles.

Electrical activation of the DpSC elicits fear-like behaviors in rats [11–13]. The DpSC and its immediately subjacent area, including the deep mesencephalic reticular formation and the deep mesencephalic gray, have been suggested as the relay station via which the amygdala facilitates startle [4,5,9,10]. In the present study, single-pulse stimulation of the DpSC could evoke startle-like responses. High-frequency tetanic stimulation of the DpSC markedly enhanced the startle-like EMG responses to ipsilateral Ce stimulation, and only slightly enhanced the EMG responses to contralateral Ce stimulation. This ipsilateral/contralateral difference is consistent to previous data indicating that the ipsilateral rostrolateral midbrain is more critical than the contralateral one in mediating startle-like responses evoked by unilateral amygdala stimulation [4,5]. Although the SC can show endogenous LTP in response to tetanic stimulation [17], it is not clear at this time how tetanic stimulation of the DpSC led to the potentiation of the amygdala-induced EMG responses seen here. This motor-output potentiation can be used to study the underlying neural plasticity induced by tetanic DpSC stimulation.

The EMG responses to IC stimulation are less plastic following DpSC tetanic stimulation than those to ipsilateral Ce stimulation. Tetanic DpSC stimulation augmented the

EMG responses to ipsilateral IC stimulation only to a small degree, and did not significantly change the responses to contralateral IC stimulation. Although the DpSC receives direct axonal projections from the ipsilateral external cortex of the IC [14], the present results suggest that the circuits by which IC outputs facilitate startle are not the same as those from the amygdala.

The most striking finding of the present study is that the Ce-induced startle-like responses can be markedly enhanced by tetanic stimulation of the ipsilateral DpSC. This mesencephalic synaptic relay station in the descending pathway from the amygdala to the primary startle circuit may allow for further interactions between fear outputs mediated by the amygdala and approach/avoidance outputs mediated by the DpSC. Investigation of the plasticity of this relay station would be important for understanding the dynamic processes of fear-modulated orientation and startle responses.

CONCLUSION

Unilateral electrical stimulation of either the Ce or the IC evoked unconditional startle-like responses at short latencies, suggesting functional connections of these two structures with the startle circuits. High-frequency unilateral tetanic stimulation of the DpSC had a strong enhancing effect on the startle-like EMG responses to ipsilateral Ce stimulation, but a smaller enhancing effect on the responses to contralateral Ce stimulation and those to ipsilateral IC stimulation, and even less effect on the responses to contralateral IC stimulation. The functional plasticity in the startle-like EMG responses to ipsilateral Ce stimulation following tetanic DpSC stimulation, therefore, provides a model for studying the neural substrates of emotional expression and learning.

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