Startle Responses to Electric Shocks: Measurement of Shock Sensitivity and Effects of Morphine, Buspirone and Brain Lesions

Der-Yow Chen, Shao-Hsuan Ho and K. C. Liang

Department of Psychology
National Taiwan University
Taipei 106, Taiwan, ROC

Abstract

The present study developed a new protocol to assess shock sensitivity in rats. Male Wistar rats were subjected to footshock stimuli ranging from 0 to 1.6 mA (0.1 s) in a startle apparatus and startle responses elicited by shocks were measured. Acoustic stimuli (95, 105, or 115 dB) were dispersed within the shock series serving as a control measurement of motor performance. Results indicated that the magnitude of shock startle responses significantly increased with the shock intensity in a linear trend. Morphine (8.0 mg/kg) and buspirone (1.0, 2.5, or 5.0 mg/kg), both of which possessing analgesic effects, depressed shock startle but had no such effect on acoustic startle. The effect of morphine was readily reversed by pretreatment of naloxone (1.0 mg/kg). To investigate the neural basis underlying this response, radio-frequency lesions of various structures implicated in processing of nociceptive or aversive information were undertaken. Lesions of the ventroposterior thalamic nucleus, insular cortex, or amygdala decreased startle reactivity to electric shocks but not to acoustic stimuli. Lesions of the anterior cingulate gyrus or medial prefrontal cortex, while altered the reactivity to acoustic stimuli, had no effect on the shock-elicited startle. These results suggested that the amplitude of startle in response to electric shocks provide a quantitative measurement of shock sensitivity within an extended range of stimulus intensities. Performing this response may engage the the central nociceptive pathway.

Key Words: naloxone, ventral posterior nucleus of thalamus, amygdala, insular cortex, nociception, acoustic startle

Introduction

Electric shocks are often adopted in learning tasks to generate aversive motivation. In classical conditioning, shock is one of the most frequent-used unconditioned stimuli (US) applied to various parts of the body (42, 59, 62). In operant conditioning, electric shocks are often utilized too. For example, a brief foot shock is dispensed to punish a rat for entering a dark chamber in the inhibitory avoidance task (4, 44-46). In the active avoidance task, avoidance or escaping responses to a warning signal are often negatively reinforced by contingent footshocks (1, 2, 35, 40). Shocks are employed to study fear and anxiety as well (17). In the conditioned emotional response (CER) paradigm, stimuli associated with shocks become capable of suppressing ongoing operant or consummatory behavior (32, 70). In the fear-potentiated startle paradigm, a neutral stimulus, e.g. a light, paired with shocks could subsequently generate fear responses and thus increase the startle response to noise bursts (14, 24, 41, 64, 72).

One important advantage of employing shock in learning tasks is its efficiency in altering behavior. For example, rats learned to avoid entering a shocked chamber in a single training trial, and this memory could last for months (34, 60). The rapid acquisition curve in these aversive learning tasks allows precise timing of the acquisition and consolidation processes, and thus renders these tasks apt for studying the neural mechanisms underlying memory processes. Accordingly, electrical or chemical treatments are

administered either before or shortly after training and the effect on acquisition or memory retention is observed respectively. However, as noted by previous studies (51), any treatment applied prior to training might affect not only the acquisition process per se, but also sensory, motor or motivational processes. Therefore, evaluating whether a pretraining treatment affecting retention has any influence on sensorimotor factors, such as shock sensitivity, becomes essential for ascribing a correct interpretation to the observed results.

A further advantage of employing the electric shock in behavioral tasks may lie on the quantifiable nature of this stimulus in comparison to other forms of stressful events, such as cold or immobilization. By changing the intensity or duration of the shock administered, an experimenter could easily control the extent of aversion in the stimulus, and hence may systematically alter the degree of learning and strength of the memory trace. However, this advantage has been largely compromised by lack of quantifiable measurement of the shock-induced response. In rats, sensitivity to electric shocks was often assessed by the flinch-jump test (3, 8, 13): Reactions to shocks were categorized into "flinch" and "jump" responses, and the threshold intensity to evoke each category of responses was calculated. This test could be criticized for its reliance on subjective observation. Assignment of a response by the experimenter would be a source of potential bias and renders the reliability of this measure questionable. Shock reactivity has also been assessed by a shock titration task in some cases (7, 12, 22, 57). In this task, rats had to press a lever to terminate a shock and decrease the shock intensity of the next trial, otherwise the shock intensity would increase gradually. However, this task in essence measures shock tolerance rather than shock sensitivity. Further, its performance relies on an acquired operant behavior, and to differentiate in this paradigm the influence of any treatment on shock responsiveness per se and on learning/memory processes would be very difficult, if not totally impossible.

In view of the above limitations in the shock sensitivity tasks presently available, it is imperative to develop a protocol which yields a quantitative measure of shock responsiveness with less or no subjective judgment. Brief and sudden appearance of stimuli in various sensory modalities, including light, noise burst, air puff, and electric shock, elicits a reflexive startle response in many species of animals (for a review, see 16). Quantified measurement of the startle amplitude is available and has been shown to be related to the stimulus intensity (16). In Experiment I, we exploited this property and developed a procedure utilizing the amplitude of startle to different intensities of shock as a measure of the sensitivity to this type of stimulus. In order to discriminate changes from shock sensitivity per se and those from startle ability in general, brief noise bursts were intermingled within a shock session. If a treatment affects startle reactivity in general, then startle responses elicited by both shock and acoustic stimuli would be altered to the same degree. On the other hand, if a treatment affects shock sensitivity specifically, then the shock-induced startle would be influenced, but the acoustic startle would not.

We also pursued the relevance to nociceptive functions in this response by examining whether the shock sensitivity measured as such could be affected by analgesic agents such as morphine or buspirone (9, 21, 27). Several recent studies addressed the central pathways involved in processing nociceptive or aversive events including electric shocks (64). In contrast to the well-delineated neural pathways underlying acoustic startle (20, 43), the neural circuitry underlying shock-elicited startle remains largely obscured. Experiment II of this study was designed to address this issue by examining the effects, on shock-elicited startle, of lesioning various brain areas implicated in processing painful or aversive experience.

General Methods

Subjects

Male Wistar rats weighing 350 to 450 grams were used in this study. After receipt from the National Experimental Animal Breeding Center, they were housed individually in air-conditioned vivariums with food and water continuously available. Throughout the study, a 12:12 hr light-dark cycle was maintained with lights on at 12:00 noon. Behavioral tests were always carried out in the light phase. The use of animal subjects in experiments abided by approved by Guidelines for Care and Use of Laboratory Animals.

Apparatus

The startle response was measured in a commercialized startle apparatus (San Diego Instrument, San Diego, U.S.A.). The animal was constrained in a Plexiglas cylindrical tube (length 20 cm, diameter 10 cm) with a vibration sensor attached to the base. Each tube was enclosed in a ventilated, sound-attenuating cabinet (length 38 cm, width 38 cm, and height 55 cm). The acoustic startle stimuli were high-intensity white noise bursts delivered by a speaker 30 cm above the animal. The shock stimuli were square-wave direct currents generated from a programmable shocker (TI 30, Coulburn Instrument, San Diego, U.S.A.). Scrambled shocks were delivered to a grid floor consisting eight metal rods inserted

inside the cylinder. The startle response was measured by the vibration sensor for a period of 200 ms after initiation of the stimulus. The vibration was transduced into voltage, then digitized and recorded by an IBM-PC compatible computer for further analyses. The startle amplitude of each trial is defined as the maximal vibration within the 200 ms.

Procedure

Matching. At the beginning of each experiment, rats were first matched for their startle performance. Briefly, animals were placed into the startle apparatus with a continuous 55 dB background noise. Five minutes later, 30 noise bursts (40 ms in duration) with a 30-second inter-stimulus interval (ISI) were presented. The intensitie were 95, 105, and 115 dB with 10 noise bursts at each level . They were presented in a balanced and quasi-random order. The mean startle amplitude across the 30 noise-burst trials was calculated for each animal and was used to assign rats into various groups such that different groups had comparable mean startle amplitude.

Startle Testing. In Experiment I, startle testing commenced one day after the matching. After receiving injections of saline or various drugs, rats were held in their home cage for 5 minutes, then placed into the startle apparatus with a continuous 55 dB background noise. After a 5-minute acclimation period, 90 startle trials were presented with an inter-trial interval of 30 s. Two types of stimuli were dispensed to elicit startle: One type of stimuli contained 9 different intensities of electric shocks ranging from 0 to 1.6 mA with an incremental step of 0.2 mA, and a duration of 0.1 s. The other type was white noise bursts as described in the matching procedure. Each session contained three blocks of trials, and each block consisted of 2 different series. Each series was composed of 6 acoustic trials (2 trials at each sound level) followed by 9 shock trials (1 trial at each shock intensity). Different intensities for each stimulus modality were presented in a semi-random order within the separated phases of a series. The total time elapsed for a test session was 50 minutes including the acclimation period.

In Experiment II, the testing procedure followed that of Experiment I with slight modifications. A session also contained 3 blocks, but each block contained one rather than two series of trials. Therefore, a session consisted a total of 45 trials. The elapsed time was thus 28 minutes for a session.

Drugs and Drug Administration

Morphine sulfate (Sigma, St. Louis, MO, U.S.

A.), buspirone (Sigma, St. Louis, MO, U.S.A.), naloxone (RBI, Natick, MA, U.S.A.), and m-chlorophenylpiperazine (mCPP) (Tocris, Bristol, U.K.) were dissolved into saline shortly before administration. All injections were administered intraperitoneally. The employed doses of morphine (2.0, 4.0, and 8.0 mg/kg), buspirone (1.0, 2.5, and 5.0 mg/kg), naloxone (0.1, 1.0, and 10.0 mg/kg), and mCPP (1.0, 3.0, and 10.0 mg/kg) were based on previous findings.

Radio-Frequency Lesions

In Experiment II, animals subjected to lesion surgeries were first injected with 0.3 mg/kg atropine sulfate (Sigma, St. Louis, MO, U.S.A.) and 10 minutes later followed by 50 mg/kg sodium pentobarbital. After fully anaesthetized, they were placed onto a stereotaxic instrument (DKI-900, Kopf, U.S.A.) and the skull was exposed. Radio-frequency lesions in different groups of rats were made by a thermal-coupled or bipolar lesion electrode inserted into the intended regions according to the coordinates of a rat brain atlas (58). In three groups of rats, a thermal-coupled electrode was inserted into the medial prefrontal cortex (mPFC: AP +2.7 mm, ML ±1.0 mm, DV -4.0 mm, n=8), anterior cingulate cortex (ACC: AP +0.5 mm, ML ±0.6 mm, DV -2.5 mm, n=8), or insular cortex (IC: AP +2.7 mm, ML ±3.1 mm, DV -6.6 mm, n=7). For two additional groups of rats, a bipolar electrode constructed by twisted stainless steel wires, insulated except at the cross section of the cut tips, was aimed at the amygdala (AMY: AP -2.7 mm, ML ±5.0 mm, DV -9.0 mm, n=13), or ventroposterior nucleus of the thalamus (VPN: AP -2.0 mm, ML ±3.1 mm, DV -6.7 mm, n=10). To approach the insular cortex without tearing the overlying cortical tissues, the electrode was inserted obliquely at an angle of 15 degree to the para-sagittal plane. To produce lesions, the lesion generator (Model RFG-4000, Radionics, Burlington, MA, U.S.A.) was turned on for 30 to 50 seconds with the thermal-coupled electrode, or 10 seconds with a current of 8 mA through the bipolar electrode. A combined sham-operated group (n=12) received the surgical procedures including insertion of electrodes into one of the target areas except that the lesion generator was not turned on. After a recovery period of at least one week, rats were subjected to startle testing.

Histology

At the end of Experiment II, lesioned rats were anesthetized with an overdose of sodium pentobarbital and perfused with physiological saline followed by 10% formalin. Brains were removed and stored in a
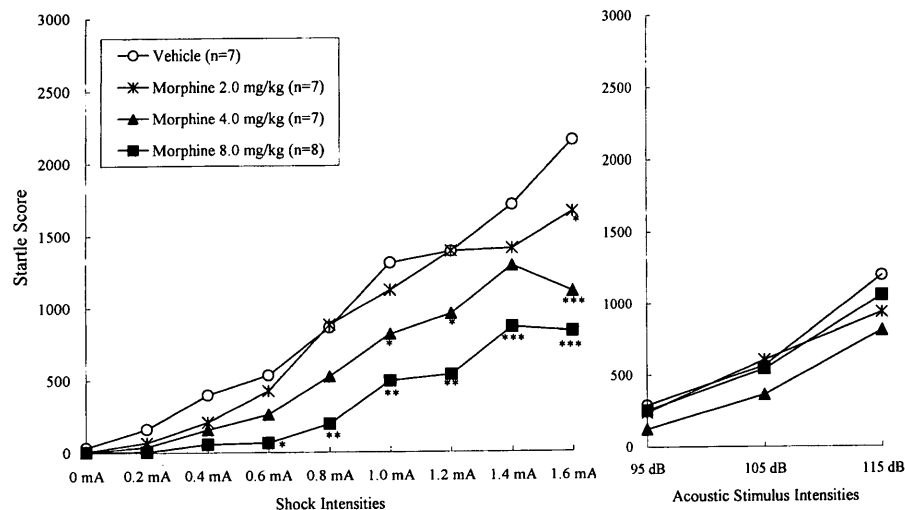
Fig. 1. The effects of morphine on shock startle (left panel) and acoustic startle (right panel). ***p<.001, **p<.01, *p<.05, significantly lower than that of the control at the specific stimulus intensity.

formalin solution containing 40% sucrose for at least 2 days. Coronal sections (40 μm thick) were cut through lesion sites on a frozen microtome under - 20°C. The slices were mounted on gelatin-coated slides, and stained with thionin. The lesion area was evaluated and plotted on coronal plates from the atlas of Paxinos and Watson (58) under a microscope.

Data Analyses

The mean startle amplitude for each stimulus modality and intensity within various blocks of the test session was calculated for each subject and these data were analyzed by analyses of variance (ANOVA). Trend analyses were performed to examine whether the startle response increased with shock intensity and post-hoc Scheffe tests were conducted for individual group comparisons. In Experiment I, the experimental design involved one between-subject variable, "Drug", and two within-subject variables, "Stimulus Intensity" and "Block". Individual three-way ANOVAs were conducted separately for shock startle and acoustic startle. In Experiment II, the mean amplitude of startle responses collapsed from all blocks was calculated as the dependent variable. The experimental design involved one between-subject variable, "Lesion", and one within-subject variable, "Stimulus Intensity". Individual two-way ANOVAs were conducted separately for shock startle and acoustic startle.

Results

Experiment I: Shock Sensitivity Measured by Startle and Effects of Morphine and Buspirone

Four groups of rats received vehicle, or morphine at a dose of 2.0, 4.0, or 8.0 mg/kg before the startle test. Figure 1 shows the mean amplitude of startle responses elicited by footshocks (left panel) or acoustic stimuli (right panel). The data showed that morphine suppressed shock startle dose-dependently but had no effect on acoustic startle. Two three-way ANOVAs (Drug × Stimulus Intensity × Block) on shock or acoustic startle showed the following results: Morphine significantly attenuated shock startle $(F(3, 25)=5.87, p<.01)$, and the Drug × Shock Intensity interaction was also significant $(F(24, 200)=3.00, p<.001)$. Post-hoc analyses showed that morphine at a dose of 8.0 mg/kg significantly suppressed shock startle, especially at high intensities of shocks (from 0.6 to 1.6 mA, all ps<.05). In contrast, injections of morphine failed to induce a significant main effect or interaction effect on acoustic startle (all Fs<1, ns). For both shock and acoustic startle, the main effect of Stimulus Intensity was significant $(F(8, 200)=94.25, p<.001$, for shock data; $F(2, 50)=53.03, p<.001$, for acoustic data). A trend analysis suggested that shock startle increased with the shock intensity in a linear trend $(F(1, 28)=127.76, p<.001)$. Post-hoc analyses indicated that the startle responses to various intensities of shock were different among themselves except for the pairs elicited by adjacent intensities (all ps<.05). In addition, acoustic startle responses elicited by three intensities of sounds were also significantly different from each other (all ps<.01). The main effect of Block and its related interactions were not significant (all Fs<1, ns).

Four groups of rats received vehicle, or naloxone at a dose of 0.1, 1.0, or 10.0 mg/kg before the startle testing session. Figure 2 shows the mean startle responses elicited by footshock (left panel) or acoustic stimuli (right panel). The data showed that naloxone failed to produce a discernible effect on shock or
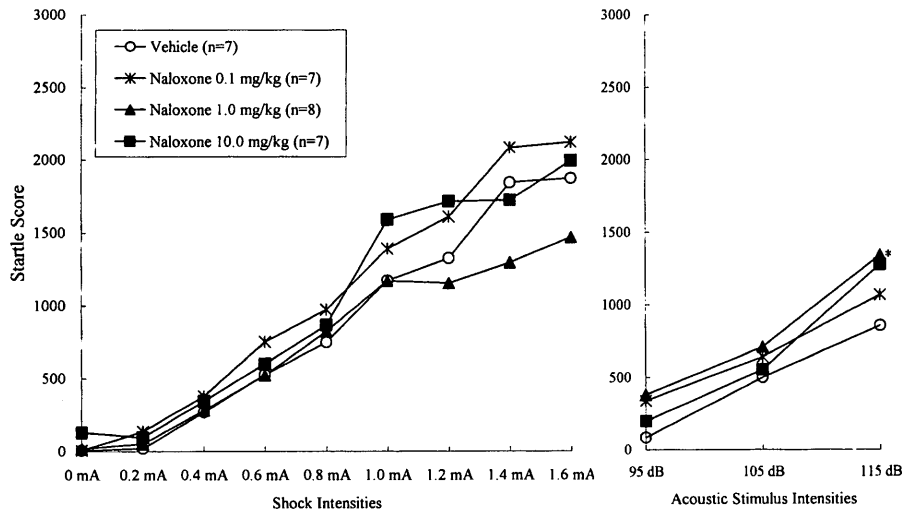
Fig. 2. The lack of effect of naloxone on shock startle (left panel) and acoustic startle (right panel). *p<.5, significantly higher than that of the control at the specific stimulus intensity.

acoustic startle. Two three-way ANOVAs (Drug × Stimulus Intensity × Block) revealed that: Naloxone failed to produce any effect in this experiment. In both shock and acoustic startle, the main effect of naloxone was not significant (both Fs<1, ns), the main effect of Stimulus Intensity was significant (F (8, 200)=73.92, p<.001, for shock data; F(2, 50)=43. 02, p<.001, for acoustic data) but the Drug × Shock Intensity interaction was not (both Fs<1, ns). Despite lack of an overall interaction, paired comparisons nonetheless showed that the 1.0 mg/kg naloxone group had higher startle responses than the vehicle group at 115 dB (p<.05). A trend analysis suggested that shock startle increased with intensity of shocks in a linear trend (F(1, 28)=123.96, p<.001). Post-hoc analyses indicated that the startle responses for various shock intensities were different among themselves except for the pair elicited by adjacent intensities (all ps<.05). In addition, acoustic startle responses elicited by the three intensities were also significantly different from each other (all ps<.01). The main effect of Block and its related interactions were not significant (all Fs<1, ns).

Additional three groups of rats were used to study antagonism of the morphine effect by naloxone in shock-elicited startle. The first group received 8.0 mg/kg morphine followed by 1.0 mg/kg naloxone; the second group received 8.0 mg/kg morphine followed by saline, and the third group received saline in both injections. The two injections were separated by 15 minutes. Rats were placed into the startle apparatus 15 minutes after the naloxone injection. The startle responses elicited by shock and acoustic stimuli are shown in Figure 3. Morphine depressed the shock-elicited startle as it did in the previous experiment, and this effect was reversed by naloxone.

Two three-way ANOVAs (Drug × Stimulus Intensity × Block) showed the following results: The main effect of Drug on shock startle approached statistical significance (F(2, 26)=3.00, p=.06), yet the Drug × Shock Intensity interaction was significant (F(16, 208)=2.93, p<.001). Post-hoc analyses showed that morphine alone suppressed shock startle, especially at high shock intensities (from 1.0 to 1.6 mA, all ps<.05), and this suppression effect of morphine was significantly attenuated by naloxone (all ps<.05). For the acoustic startle, no overall statistical significance was detected in the Drug main effect or the Drug × Sound Intensity interaction effect (F(2, 26)=1.62 and F(4, 52)=0.43, respectively; p>.05). However, the morphine+ naloxone group appeared to show higher startle than the other two groups and paired comparisons revealed significant group differences at 105 and 115 dB of sound levels (all ps<.05). For both shock and acoustic startle, the main effect of Stimulus Intensity was significant (F(8, 208)=61.86, p<.001, for shock data; F(2, 52) =42.39, p<.001, for acoustic data). A trend analysis revealed that shock startle increased with shock intensity in a linear trend (F(1, 28)=84.22, p<.001). Post-hoc analyses indicated that the startle responses for various shock intensities were different among themselves except for the pair elicited by adjacent intensities (all ps<.05). In addition, the startle responses elicited by the three sound levels were also significantly different from each other (all ps<.01). The main effect of Block and its related interactions were not significant (all Fs<1, ns).

Four additional groups of rats receiving vehicle, or buspirone at the dose of 1.0, 2.5, or 5.0 mg/kg before the startle testing session. Figure 4 shows the mean startle responses elicited by footshock (left
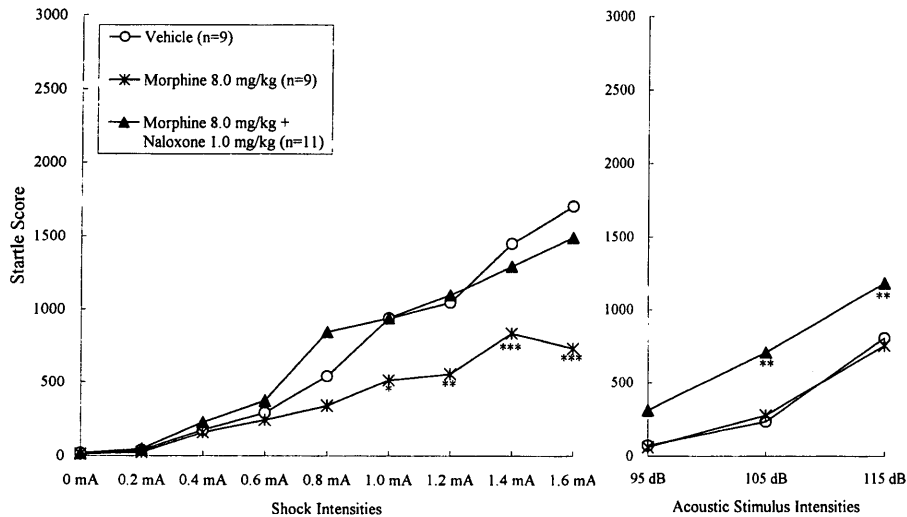
Fig. 3. Attenuation of the morphine effect by naloxone in shock startle (left panel) and acoustic startle (right panel). ***p<.001, significantly lower than that of the control at the specific stimulus intensity.
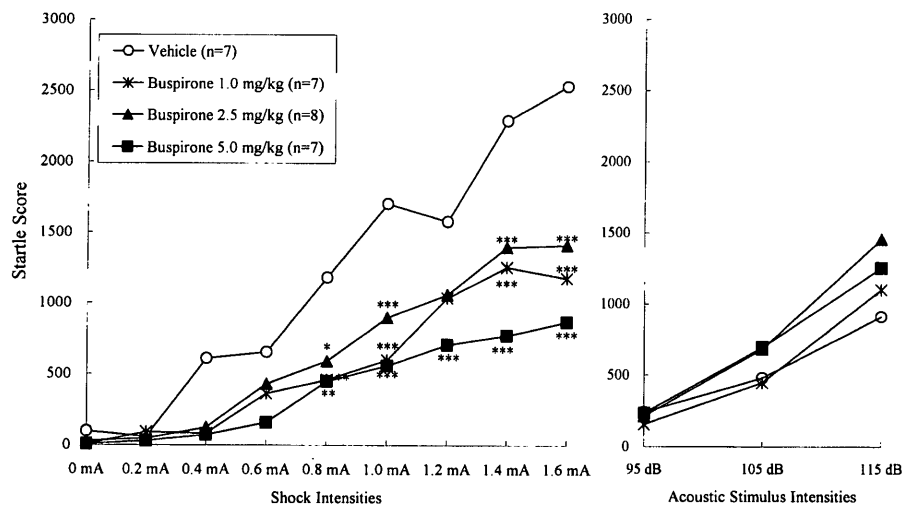

Fig. 4. The effects of buspirone on shock startle (left panel) and acoustic startle (right panel). ***p<.001, **p<.01, *p<.05, significantly lower than that of the control at the specific stimulus intensity.

panel) or acoustic stimuli (right panel). The data showed that buspirone suppressed shock-induced startle dose-dependently but had no effect on acoustic startle. Two three-way ANOVAs (Drug × Stimulus Intensity × Block) showed the following results: For shock startle, the main effect of Drug was statistically significant (F(3, 25)=6.13, p<.01), and so was the Drug × Shock Intensity interaction (F(24, 200)=3.18, p<.001). Post-hoc analyses showed that all doses of buspirone significantly suppressed shock startle at high shock intensities (from 0.8 to 1.6 mA, all ps<.05). On the other hand, for acoustic startle the Drug effect was not significant (F(3, 25)<1), and neither was the Drug × Sound Intensity interaction (F(6, 50)=1.05, p>.05). For both shock and acoustic startle, the main effect of Stimulus Intensity was significant (F(8, 200)=63.52, p<.001, for shock data;

F(2, 50)=63.96, p<.001, for acoustic data). A trend analysis suggested that shock-induced startle increased with intensity of shocks in a linear trend (F(1, 28)= 93.76, p<.001). Post-hoc analyses indicated that the startle responses for various shock intensities were different among themselves except for the adjacent couples (all ps<.05). In addition, the startle responses elicited by three intensities of acoustic stimuli were also significantly different from each other (all ps<.01). The main effect of Block and all related interactions were not significant (all Fs<1, ns).

To demonstrate that acoustic startle in this paradigm was sensitive to manipulation, four groups of rats received before the startle test injections of vehicle, or 1.0, 3.0, or 10.0 mg/kg of mCPP, a drug known to affect acoustic startle (18, 49, 67). Figure 5 shows the mean startle responses elicited by
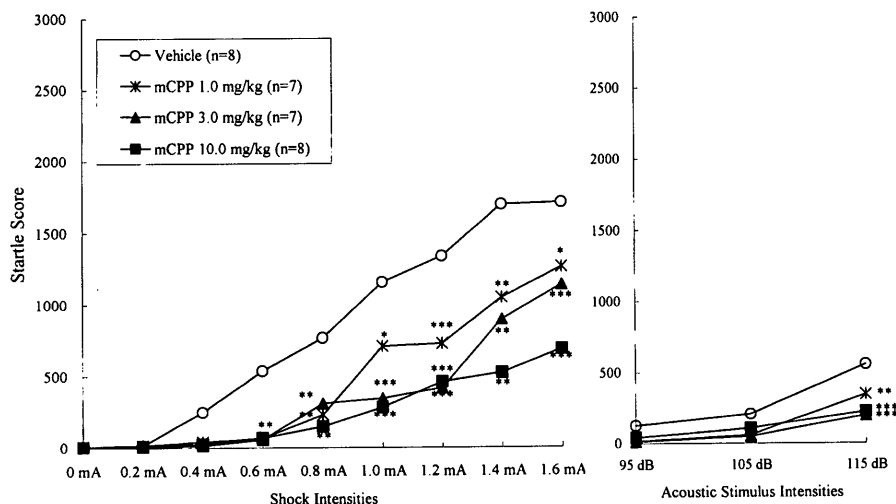
Fig. 5. The effects of mCPP on shock startle (left panel) and acoustic startle (right panel). ***p<.001, **p<.01, *p<.05, significantly lower than that of the control at the specific stimulus intensity.

footshock (left panel) or acoustic stimuli (right panel). The data showed that mCPP suppressed both acoustic and shock-induced startle in a dose-dependent manner. Two three-way ANOVAs (Drug × Stimulus Intensity × Block) showed the following results: For shock startle, the main effect of Drug was statistically significant $(F(3, 26) = 8.36, p<.001)$, and so was the Drug × Shock Intensity interaction $(F(24, 208)=4.04, p<.001)$. Post-hoc analyses showed that all doses of mCPP significantly suppressed the shock startle at high shock intensities (from 0.6 to 1.6 mA, all ps<.05). For acoustic startle, the Drug main effect was also significant $(F(3, 26)=3.01, p<.05)$, but the Drug × Sound Intensity interaction was not $(F(6, 52) =1.64, p>.05)$. Post-hoc analyses showed that all doses of mCPP significantly suppressed the startle response at the 115 dB sound level (all ps<.01). For both shock and acoustic startle, the main effect of Stimulus Intensity was significant $(F(8, 208)=78.29, p < .001$, for shock data; $F(2, 52)=31.28, p<.001$, for acoustic data). A trend analysis suggested that shock-induced startle increased with intensity of shocks in a linear trend $(F(1, 29)=90.07, p<.001)$. Post-hoc analyses indicated that the startle responses for various shock intensities were different among themselves except for the adjacent pairs (all ps<.05). In addition, the startle responses at 115 dB were also significantly higher than those at other sound levels (both ps<.001). The main effect of Block and its related interactions were not significant (all Fs<1, ns).

Experiment II: Effects of Brain Lesions on Shock Sensitivity Measured by Startle

Histological reconstructions of various brain

lesions in representative cases are shown in Figure 6. Rats without adequate damage of the target tissues were eliminated from the data analysis. Thus, a total of 43 rats bearing lesions in one of the several target regions and 12 sham rats were used for the final data analysis. Since the previous experiment failed to show a significant main or interaction effect of Block main or interaction effect responses were relatively stable within a testing session. Thus, the startle responses in this experiment were collapsed across all blocks and an overall mean amplitude was calculated as the dependent variable. Further, since rats bearing sham lesions at different sites did not significantly differ in startle scores, they were collapsed into a combined sham group.

The effects of brain lesions on the startle responses are summarized in Figure 7, which showed that lesions of the VPN, IC, and AMY decreased shock startle. In contrast, lesions of the ACC or mPFC had no effect. Overall two-way ANOVAs (Lesions × Stimulus Intensity) were first conducted for on shock and acoustic startle. For both sets of data, the main effect of Lesion, or Stimulus Intensity and the interaction effect between the two were all significant (for shock data: $F(5, 55)=5.63$, $F(5, 440) =59.96$, and $F(40, 440)=3.14$, all ps<.001, respectively; for acoustic data: $F(5, 55)=2.54, p<.05, F(2, 110)=84. 84, p<.001, F(10, 110)=1.94, p<.05$; respectively).

In order to better characterize the role of each target region on shock sensitivity, the data were further analyzed by individual two-way ANOVAs comparing each lesioned group with the combined sham group on both types of startle. Lesions of the VPN depressed shock startle in rats: Shock startle in the lesioned group was slightly lower than that of the combined control but the difference failed to reach statistical
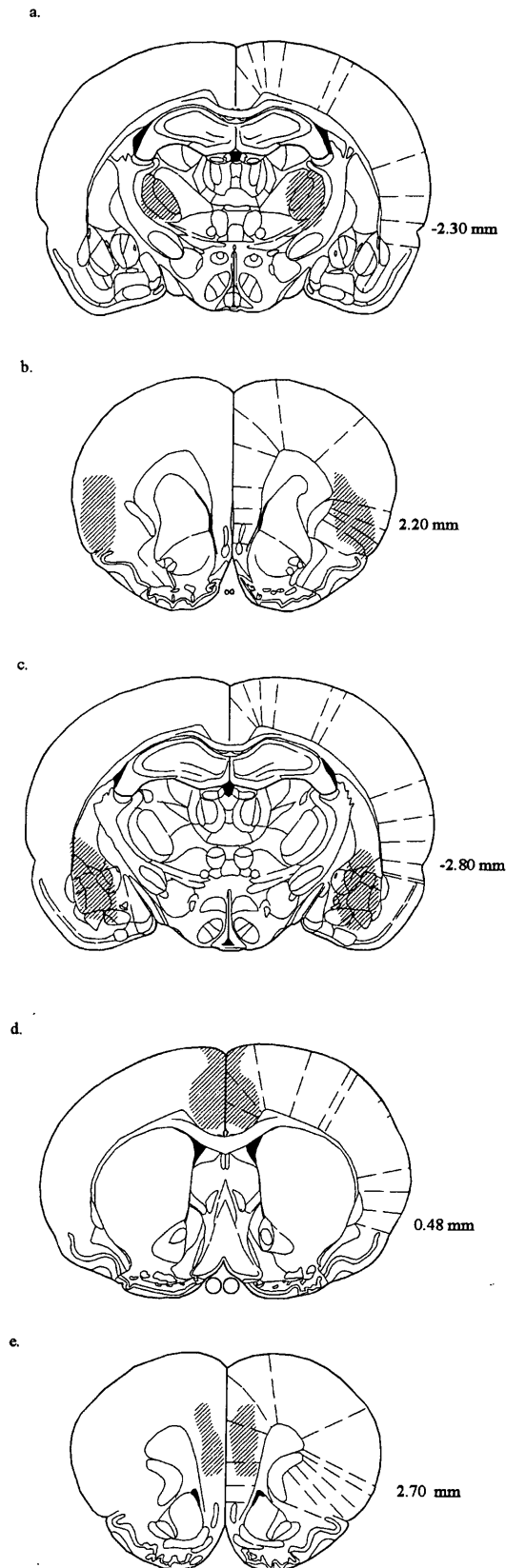
Fig. 6. Histological reconstructions of representative lesions on coronal plates from the atlas of Paxinos and Watson (58): a. ventroposterior nucleus of thalamus (VPN); b. insular cortex (IC); c. amygdala (AMY); d. anterior cingulate cortex (ACC); e. medial prefrontal cortex (mPFC).

significance $(F(1, 18)=2.69, p=0.13)$, yet the Lesion × Shock Intensity interaction was significant $(F(8, 144) =2.75, p<0.01)$ and post-hoc analyses showed that the startle responses to 1.4 and 1.6 mA shocks were significantly lower in the VPN lesioned group (all ps <.01), suggesting responsiveness to higher shocks. In contrast, the acoustic startle of the VPN lesioned group was significantly higher than that of the combined control $(F(1, 18)=5.02, p<.05)$ and post-hoc analyses showed that the startle responses to 105 and 115 dB noise bursts were significantly higher in the VPN lesioned group (both ps<.001), suggesting enhanced responsiveness to louder sounds.

Lesions of the IC also depressed the shock-elicited startle in rats: The shock-elicited startle response of the lesioned group was lower than that of the combined control $(F(1, 17)=5.81, p<.05)$. The Lesion × Shock Intensity interaction was significant $(F(8, 136)=3.96, p<0.001)$, and post-hoc tests showed that the startle responses were lower in the IC lesioned group at shock intensities from 1.2 to 1.6 mA (all ps <.001), suggesting that the lesions depressed responsiveness to higher shocks. The acoustic startle response in the IC lesioned group was not significantly different from that in the combined control $(F(1, 17) <1)$.

Lesions of the AMY depressed shock startle as well: Shock startle response of the AMY lesioned group was slightly lower than that of the combined control but failed to reach statistical significance $(F (1, 22)=2.73, p=0.12)$, yet the Lesion × Shock Intensity interaction was significant $(F(8, 176)=2.51, p<.05)$ and post-hoc analyses showed that startle responses at 1.2 and 1.6 mA were significantly lower in the AMY lesioned group (both ps<.001), suggesting that the lesions depressed responsiveness to higher shocks. In contrast, the acoustic startle in the AMY lesioned group was significantly higher than that of combined control $(F(1, 22)=5.14, p<.05)$, and post-hoc analyses showed that the startle responses to all sound intensities were significantly higher in the AMY lesioned group (all ps<.001), indicative of elevated responsiveness to sounds by the lesions.

The ACC lesioned group showed slightly higher shock startle than the combined control but the difference failed to reach statistical significance $(F (1, 18)=3.87, p<0.10)$, and neither did the Lesion × Shock Intensity interaction $(F(8, 144)=1.57, p>.05)$. However, paired comparisons revealed that the ACC lesioned group displayed higher startle responses to 1.0 and 1.6 mA shocks than that of the combined control $(F(1, 162)=11.18, p<.01; F(1, 162)=25.30, p<.001)$. On the other hand, acoustic startle was higher in the ACC lesioned group $(F(1, 18) = 7.44, p<.05)$, and post-hoc analyses showed that the startle responses to all sound intensities were significantly
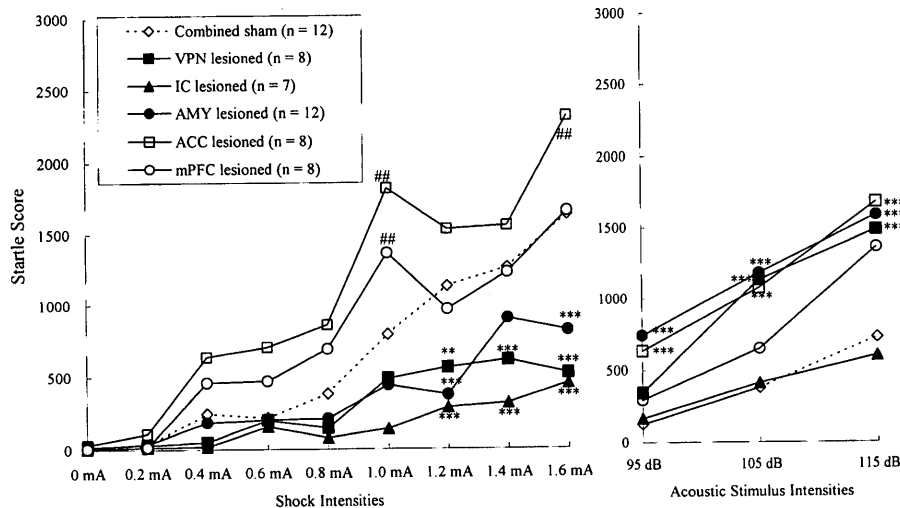
Fig. 7. The effects of brain lesions on shock startle (left panel) and acoustic startle (right panel). Six groups of rats received sham lesions, or lesions of the VPN, IC, AMY, ACC, or mPFC. ***p<.001, **p<.01, significantly lower than that of the combined control at the specific stimulus intensity. ###p<.001, ##p<.01, significantly higher than that of the combined control at the specific stimulus intensity.

higher in the lesioned group (all ps<.001).

For both shock startle and acoustic startle, the overall mPFC main effect was not significant (F(1, 18)=0.28 or 1.24, p>.05; respectively), nor was the Lesion × Shock Intensity interaction (F(8, 144) =0.85, F(8, 160)=1.30, p>.05; respectively). However, paired comparisons revealed that the mPFC lesioned group displayed higher startle responses to 1.0 mA of shocks than the combined control group (F'(1, 162) =7.59, p<.01).

Discussion

The present study documented a protocol for assessing shock sensitivity by measuring shock-induced startle. This protocol yields quantitative data not only devoid of subjective judgments but also sensitive to changes in shock sensitivity, as attested by the findings that the startle amplitude in most cases increased linearly with the shock intensity within the tested range. In addition, this novel procedure could simultaneously assess the startle response to acoustic stimuli. This built-in control helps to determine whether effects on shock-induced startle, if observed, are due to alteration in shock sensation specifically or startle reactivity in general. Thus, in comparison with other available tasks for the same purposes (3, 8, 13), this new paradigm offers a better solution.

According to the present study, various pharmacological agents or brain lesions reduced shock reactivity. In general, these effects did not reach statistical significance until a medium- or high-intensity shock was administered (0.6 to 1.6 mA). It is worth noting that most available data reported a flinch threshold at 0.25 to 0.30 mA, a jump threshold at 0.35 to 0.45 mA, that might be well below the

intensity upon which manipulations in this study could show an effect. Thus, a given treatment not changing the flinch-jump threshold does not necessarily guarantee the integrity of shock sensitivity. Accordingly, lack of influences of some pretraining administered treatments in a flinch-jump test, such as intra-AMY infusion of an NMDA receptor antagonist AP5 (38, 39), provides no warranty for attributing the observed effect to inflicted acquisition, particularly if the shock administered in the learning task was well beyond the jump threshold. Our present findings thus call for careful interpretation of the findings produced by any pretraining treatments on memory, such as buspirone injections or AMY lesions (44).

Previous studies have reported that the startle reflex underwent rapid habituation in responses to a series of acoustic or tactile stimuli (15, 25). However, the present results showed that the amplitude of startle responses to stimuli of the same intensity was stable across 3 blocks of trials as indicated by lack of any Block × Stimulus Intensity interaction in all analyses of Experiment I. The lack of habituation to electric shocks may be due to the fact that not only the intensity of shocks varied randomly from trial to trial, but also that acoustic stimuli dispersed within the shock series. These conditions may result in dishabituation as predicted by the fundamental properties of habituating processes (68). In addition, habituation occurs most often in response to repetition of innocuous stimuli. For recurring noxious and intense stimuli such as electric shocks, decrease of responsiveness would be non-adaptive. As a matter of fact, previous reports have shown that presentation of peripheral noxious stimuli sometimes caused sensitization in startle responses (31, 63). Such sensitization may obliterate any concurrent habituation

caused by repeated stimulation.

Results of Experiment I showed that 8.0 mg/kg morphine attenuated the startle response elicited by high-intensity shocks (from 0.6 to 1.6 mA), but had no effect on the startle response elicited by acoustic stimuli. Furthermore, naloxone at a dose of 1.0 mg/ kg, which by itself had no effect on shock reactivity or acoustic startle, readily antagonized the depressive effect of morphine, suggesting that opioid μ-receptors modulate the startle reaction to shocks of medium to high intensities. Previous studies showed that neither morphine nor naloxone altered the baseline of acoustic startle (14), which was in general replicated by our findings. However, our data did show a slight increase of acoustic startle, particularly at high sound intensity, after injections of 1.0 mg/kg naloxone either alone or in conjunction with morphine. It might be argued that attenuation of the morphine effect by naloxone is due to a general startle-enhancing effect of naloxone and bears no relevance to receptor antagonism. Such explanation was implausible in view of that rats given 1.0 mg/kg naloxone alone displayed no elevated startle to electrical shocks (Fig. 2).

The lack of a prominent effect of morphine on acoustic startle could not be attributed to poor sensitivity of this measure in our paradigm, because we demonstrated that acoustic startle in our paradigm could be suppressed by a $5HT_{1B/2C}$ agonist mCPP, consistent with previous findings (18, 49, 67). This drug also suppressed shock startle, the impairments in both types of startle could thus be due to a general motor deficit produced by mCPP. Alternatively, mCPP could increase reaction latencies in a tailflick test (56), and thus may have an analgesic effect of its own. While the present data failed to show a clear-cut double dissociation of the two types of startle responses pharmacologically, the findings on mCPP provide valid evidence that the acoustic startle measure in our paradigm serves as a useful internal control.

Selective depression of shock startle but not acoustic startle by morphine suggests that this response may engage the central nociceptive pathway as shock intensities reach a moderate level. Consistent with this conjecture was the finding that buspirone inhibited the shock startle at all doses tested. Again this effect was significant at shock intensities ranging from 0.8 to 1.6 mA but for acoustic startle this effect was indiscernible. The inhibitory effect of buspirone on shock reactivity could be attributed to its analgesic effect as shown in several nociceptive tasks involving thermal, mechanical or chemical stimuli (9, 27, 55). This effect could be due to a $5-HT_{1A}$ agonist action of buspirone as serotoninergic fibers from the brain stem mediate an antinociceptive action (23, 36). Additionally, buspirone has anxiolytic effects. Previous findings have shown that buspirone blocked

the expression of conditioned fear to a stimulus paired with shocks (19, 37). It is thus also likely that suppression of shock-induced emotion or arousal by buspirone contributes to lowering reactivity to noxious or painful stimuli, because affective reaction is taken to be one of the major components of subjective pain (53).

Electric shocks may be the most often used US or motivating stimulus in conditioning tasks, but until recently little available data addressed systematically the issue of how electric shocks are processed in the central nervous system. The present study examined this issue by probing some structures implicated in nociception or aversive learning. Our results showed that radio frequency lesions of the VPN, IC, or the AMY produced marked suppressive effects on startle responses to intense shocks. This effect was not due to general motor deficits as the acoustic startle of the lesioned rats was either normal (IC lesions), or even elevated (VPN or AMY lesions). In contrast, lesions of the ACC or mPFC did not depress shock-induced startle at all, and if there was any effect, these lesions might even increase shock-startle at some shock intensities. These data achieve some sort of dissociation of the anatomical circuitries underlying the two types of startle in our paradigm.

It had been suggested that the VPN, composed of a medial and a lateral sectors, serves as an important relay in the lateral pain system (74). The medial nucleus receives facial nociceptive information through afferents from the trigeminal nuclei, and the lateral nucleus receives topographical somatosensory input through the spinothalamic tract. Painful peripheral stimulation increased neural activity in the lateral nucleus of the VPN (50). In the present study, radio frequency lesions of the VPN depressed shock-induced startle at higher shock intensities which may engage nociception as such behavior could be suppressed by morphine, was consistent with the role of VPN in nociception.

Recent studies suggest that the IC is involved in somatosensory functions including sensation of pain (33). The IC receives convergent inputs from somatosensory cortices, VPN, and posterior thalamic nuclei, posterior intralaminar nuclei, and parabrachial nucleus (65, 66), all of which have been implicated in processing noxious somatosensory stimuli. Our findings that IC lesions produced a depressive effect on shock-elicited startle are consistent with such a notion. The IC was suggested to mediate opiate antinociception (11): Microinfusion of naloxone into the rostral agranular IC reversed the behavioral analgesic effect of systemic morphine in the formalin test, that was related to an increased inhibition descending to the dorsal horn neurons based on electrophysiological and immunohistochemical

evidence. How the nociceptive input to the IC resulates antinociception should be elucidated in the future.

As part of the limbic system, the AMY has long been implicated in processing of aversive experience and emotional memory (52). However, increasing attention has also been paid to its pain-modulating role. Electrolytic lesions of the medial AMY decreased animals' pain reactivity in the hot-plate and tail-flick tests (73). Intra-AMY infusion of an AMPA receptor antagonist CNQX increased flinch-jump thresholds (54). In the present study, the shock sensitivity of the AMY-lesioned rats were also depressed especially in higher shock intensities, consistent with a previous finding that the overall level of shock reactivity was lower in rats with lesions in the central AMY (31). The AMY may be involved in the endogenous antinociceptive function (29, 30, 47, 48). For example, microinjections of morphine into AMY significantly elevated the threshold of response in the flinch-jump test (61). NMDA lesions of the central AMY nucleus abolished the morphine-induced antinociception in the tail-flick test (48).

The central AMY nucleus receives nociceptive input from the parabrachial nucleus which is innervated by direct spinal nociceptive fibers (5, 6, 74). In the fear-potentiated startle task, two parallel pathways have been proposed to relay shock information into the AMY (64-66): One involves subcortical projection from the posterior intralaminar nuclei of the thalamus to the AMY, and the other involves projections from the caudal part of the insular cortex. The present findings that lesions of the VPN, IC and AMY compromised shock sensitivity suggest that the latter pathway plays a critical role for mediating shock information to modulate startle response. Whatever the route might be, our data are consistent with the neuroanatomical and neurophysiological findings that the AMY is part of the central nociceptive processing circuitry.

Abundance of evidence has suggested that pain experience activated the ACC (71), especially in chronic pain states. However, its role appears to be task-dependent because injection of lidocaine into the anterior cingulum bundle produced significant reductions in the formalin pain score, but had no effect on the foot-flick latency (69). The present study failed to show a depression effect of ACC lesions on shock-elicited startle, which suggests that ACC might not play a role in shock-induced nociception. Alternatively, the general increased in reactivity as indicated by increased acoustic startle may have masked any otherwise apparent effect of ACC lesions in depressing startle response to a sudden noxious stimulus. These two possibilities remain to be clarified in the future.

A previous study reported direct nociceptive

projection from the spinal cord to the hypothalamus and that some in these fibers coursed to the mPFC (26). While the functional role of this projection remains obscured, although stimulation of the mPFC produced analgesia (28). Accordingly, mPFC lesions should have produced hyperalgesia and increased shock startle. The present study did not yield clear and consistent results to support this prediction, although mPFC lesions did increase reactivity to 1.0 mA shock. The effect of mPFC lesions on shock sensitivity could not have been masked by any general change in startle responses, because lesions of the mPFC did not alter acoustic startle as shown by this and previous studies (10). The exact role of the mPFC in processing of electric shocks, as well as other painful stimuli, should be pursued in the future.

In summary, the present study showed that shock startle was suitable for assessment of shock sensitivity. It offers an objective and quantitative index for a wide range of shocks as opposed to the flinch-jump test. The susceptibility of this measurement to analgesic agents attests the involvement of central nociceptive pathways, particularly under high shock conditions. Therefore, this paradigm cannot only be adopted as a task for checking the effect of various treatments on reaction to electric shocks, but also be exploited to investigate the central substrates engaged by noxious stimuli. This latter work may contribute to elucidating the central mechanisms of how an aversive US forges its association with a neutral CS in the nervous system.

Acknowledgment

References

1. Aguilar, M.A., Minarro, J. and Simon, V.M. Dose-dependent impairing effects of morphine on avoidance acquisition and performance in male mice. *Neurobiol. Learn. Mem.* 69: 92-105, 1998.
2. Becker, A., Letzel, K., Letzel, U. and Grecksch, G. Kindling of the dorsal and the ventral hippocampus: effects on learning performance in rats. *Physiol. Behav.* 62: 1265-71, 1997.
3. Berge, O.G., Hole, K. and Ogren, S.O. Attenuation of morphine-induced analgesia by p-chlorophenylalanine and p-chloroamphetamine: test-dependent effects and evidence for brainstem 5-hydroxytryptamine involvement. *Brain Res.* 271: 51-64, 1983.
4. Bermudez-Rattoni, F., Introini-Collison, I., Coleman-Mesches, K.

and McGaugh, J.L. Insular cortex and amygdala lesions induced after aversive training impair retention: effects of degree of training. *Neurobiol. Learn. Mem.* 67: 57-63, 1997.

5. Bernard, J.F. and Besson, J.M. The spino(trigemino) pontoamygdaloid pathway: electrophysiological evidence for an involvement in pain processes. *J. of Neurophysiol.* 63: 473-90, 1990.

6. Bernard, J.F., Bester, H. and Besson, J.M. Involvement of the spino-parabrachio -amygdaloid and -hypothalamic pathways in the autonomic and affective emotional aspects of pain. *Prog. Brain Res.* 107: 243-55, 1996.

7. Bloss, J.L. and Hammond, D.L. Shock titration in the rhesus monkey: effects of opiate and nonopiate analgesics. *J. Pharmacol. Exp. Ther.* 235: 423-30, 1985.

8. Bonnet, K.A. and Peterson, K.E. A modification of the jump-flinch technique for measuring pain sensitivity in rats. *Pharmacol. Biochem. Behav.* 3: 47-55, 1975.

9. Bragin, E.O., Korneev, A.Y. and Vasilenko, G.F. Buspirone effect on the development of antinociceptive reactions. *Pain* 36: 257-61, 1989.

10. Bubser, M. and Koch, M. Prepulse inhibition of the acoustic startle response of rats is reduced by 6-hydroxydopamine lesions of the medial prefrontal cortex. *Psychopharmacol.* 113: 487-92, 1994.

11. Burkey, A.R., Carstens, E., Wenniger, J.J., Tang, J. and Jasmin, L. An opioidergic cortical antinociception triggering site in the agranular insular cortex of the rat that contributes to morphine antinociception. *J. Neurosci.* 16: 6612-23, 1996.

12. Craft, R.M. and Dykstra, L.A. Differential cross-tolerance to opioids in squirrel monkeys responding under a shock titration schedule. *J. Pharmacol. Exp. Ther.* 252: 945-52, 1990.

13. Crocker, A.D. and Russell, R.W. The up-and-down method for the determination of nociceptive thresholds in rats. *Pharmacol. Biochem. Behav.* 21: 133-6, 1984.

14. Davis, M. Morphine and naloxone: effects on conditioned fear as measured with the potentiated startle paradigm. *Eur. J. Pharmacol.* 54: 341-7, 1979.

15. Davis, M. Neurochemical modulation of sensory-motor reactivity: acoustic and tactile startle reflexes. *Neurosci. Biobehav. Rev.* 4: 241-63, 1980.

16. Davis, M., The mammalian startle response. In R.C. Eaton (Ed.), Neural mechanisms of startle behavior. New York: Plenum Press, pp. 287-351, 1984.

17. Davis, M. Animal models of anxiety based on classical conditioning: the conditioned emotional response (CER) and the fear-potentiated startle effect. *Pharmacol. Ther.* 47: 147-65, 1990.

18. Davis, M., Cassella, J.V., Wrean, W.H. and Kehne, J.H. Serotonin receptor subtype agonists: differential effects on sensorimotor reactivity measured with acoustic startle. *Psychopharmacol. Bull.* 22: 837-43, 1986.

19. Davis, M., Falls, W.A., Campeau, S. and Kim, M. Fear-potentiated startle: a neural and pharmacological analysis. *Behav. Brain Res.* 58: 175-98, 1993.

20. Davis, M., Gendelman, D.S., Tischler, M.D. and Gendelman, P.M. A primary acoustic startle circuit: lesion and stimulation studies. *J. Neurosci.* 2: 791-805, 1982.

21. Dennis, S.G. and Melzac, R., Comparison of phasic and tonic pain in animals. In J.J. Bonica, Liebeskind, J.C. and Albe-Fessard, D. (Eds.), Advances in pain research and therapy, Vol. 3. New York: Raven press, 1979.

22. Dykstra, L.A., Powell, K.R. and Lin, Y.P. Antinociceptive effects of the kappa opioid, U50,488: lack of modulation by 5-HT$_2$ antagonists. *Psychopharmacol.* 112: 116-20, 1993.

23. Engberg, G. A metabolite of buspirone increases locus coeruleus activity via alpha 2-receptor blockade. *J. Neural Trans.* 76:91-98, 1989.

24. Gewirtz, J.C., McNish, K.A. and Davis, M. Lesions of the bed nucleus of the stria terminalis block sensitization of the acoustic startle reflex produced by repeated stress, but not fear-potentiated startle. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* 22: 625-48, 1998.

25. Geyer, M.A. and Tapson, G.S. Habituation of tactile startle is altered by drugs acting on serotonin-2 receptors. *Neuropsychopharmacol.* 1: 135-47, 1988.

26. Giesler, G.J. Evidence of direct nociceptive projecitons from the spinal cord to the hypothalamus and telencephalon. *Seminar Neurosci.* 7: 253-261, 1995.

27. Giordano, J. and Rogers, L. Antinociceptive effects of the novel anxiolytic buspirone in three pain tests in rats. *Pain* 39: 109-13, 1989.

28. Hardy, S.G. Analgesia elicited by prefrontal stimulation. *Brain Res.* 339: 281-4, 1985.

29. Helmstetter, F.J. and Bellgowan, P.S. Lesions of the amygdala block conditional hypoalgesia on the tail flick test. *Brain Res.* 612: 253-7, 1993.

30. Helmstetter, F.J., Tershner, S.A., Poore, L.H. and Bellgowan, P.S. Antinociception following opioid stimulation of the basolateral amygdala is expressed through the periaqueductal gray and rostral ventromedial medulla. *Brain Res.* 779: 104-18, 1998.

31. Hitchcock, J.M., Sananes, C.B. and Davis, M. Sensitization of the startle reflex by footshock: blockade by lesions of the central nucleus of the amygdala or its efferent pathway to the brainstem. *Behav. Neurosci.* 103: 509-18, 1989.

32. Hoehn-Saric, R., McLeod, D.R. and Glowa, J.R. The effects of NMDA receptor blockade on the acquisition of a conditioned emotional response. *Biol. Psychiatry* 30: 170-6, 1991.

33. Ito, S.I. Possible representation of somatic pain in the rat insular visceral sensory cortex: a field potential study. *Neurosci. Lett.* 241: 171-4, 1998.

34. Izquierdo, I., Quillfeldt, J.A., Zanatta, M.S., Quevedo, J., Schaeffer, E., Schmitz, P.K. and Medina, J.H. Sequential role of hippocampus and amygdala, entorhinal cortex and parietal cortex in formation and retrieval of memory for inhibitory avoidance in rats. *Eur. J. Neurosci.* 9: 786-93, 1997.

35. Joel, D., Tarrasch, R., Feldon, J. and Weiner, I. Effects of electrolytic lesions of the medial prefrontal cortex or its subfields on 4-arm baited, 8-arm radial maze, two-way active avoidance and conditioned fear tasks in the rat. *Brain Res.* 765: 37-50, 1997.

36. Jones, S.L. Descending noradrenergic influences on pain. *Prog. Brain Res.* 88: 381-94, 1991.

37. Kehne, J.H., Cassella, J.V. and Davis, M. Anxiolytic effects of buspirone and gepirone in the fear-potentiated startle paradigm. *Psychopharmacol.* 94: 8-13, 1988.

38. Kim, J.J., DeCola, J.P., Landeira-Fernandez, J. and Fanselow, M.S. N-methyl-D-aspartate receptor antagonist APV blocks acquisition but not expression of fear conditioning. *Behav. Neurosci.* 105: 126-33, 1991.

39. Kim, M. and McGaugh, J.L. Effects of intra-amygdala injections of NMDA receptor antagonists on acquisition and retention of inhibitory avoidance. *Brain Res.* 585: 35-48, 1992.

40. Lacroix, L., Broersen, L.M., Weiner, I. and Feldon, J. The effects of excitotoxic lesion of the medial prefrontal cortex on latent inhibition, prepulse inhibition, food hoarding, elevated plus maze, active avoidance and locomotor activity in the rat. *Neuroscience* 84: 431-42, 1998.

41. Lamont, E.W. and Kokkinidis, L. Infusion of the dopamine D$_1$ receptor antagonist SCH 23390 into the amygdala blocks fear expression in a potentiated startle paradigm. *Brain Res.* 795: 128-36, 1998.

42. LeDoux, J.E., Iwata, J., Cicchetti, P. and Reis, D.J. Different projections of the central amygdaloid nucleus mediate autonomic and behavioral correlates of conditioned fear. *J. Neurosci.* 8: 2517-29, 1988.

43. Lee, Y., Lopez, D.E., Meloni, E.G. and Davis, M. A primary acoustic startle pathway: obligatory role of cochlear root neurons

and the nucleus reticularis pontis caudalis. *J. Neurosci.* 16: 3775-89, 1996.

44. Liang, K.C. Pre- or post-training injection of buspirone impaired retention in the inhibitory avoidance task: involvement of amygdala 5-HT$_{1A}$ receptors. *Eur. J. Neurosci.* 11: 1491-500, 1999.

45. Liang, K.C., Tsui, K.Y., Tyan, Y.M. and Chiang, T.C. Buspirone impaired acquisition and retention in avoidance tasks: involvement of the hippocampus. *Chin. J. Physiol.* 41: 33-44, 1998.

46. Ma, Y.L., Wang, H.L., Wu, H.C., Wei, C.L. and Lee, E.H. Brain-derived neurotrophic factor antisense oligonucleotide impairs memory retention and inhibits long-term potentiation in rats. *Neuroscience* 82: 957-67, 1998.

47. Manning, B.H. and Mayer, D.J. The central nucleus of the amygdala contributes to the production of morphine antinociception in the formalin test. *Pain* 63: 141-52, 1995.

48. Manning, B.H. and Mayer, D.J. The central nucleus of the amygdala contributes to the production of morphine antinociception in the rat tail-flick test. *J. Neurosci.* 15: 8199-213, 1995.

49. Mansbach, R.S. and Geyer, M.A. Blockade of potentiated startle responding in rats by 5-hydroxytryptamine$_{1A}$ receptor ligands. *Eur. J. Pharmacol.* 156: 375-83, 1988.

50. Mao, J., Mayer, D.J. and Price, D.D. Patterns of increased brain activity indicative of pain in a rat model of peripheral mononeuropathy. *J. Neurosci.* 13: 2689-702, 1993.

51. McGaugh, J.L. Dissociating learning and performance: drug and hormone enhancement of memory storage. *Brain Res. Bull.* 23: 339-45, 1989.

52. McGaugh, J.L., Introini-Collison, I.B., Cahill, L., Kim, M. and Liang, K.C., Involvement of the amygdala in neuromodulatory influences on memory storage. In J.P. Aggleton (Ed.), The amygdala: Neurobiological aspects of emotion, memory, and mental dysfunction. New York: Wiley-Liss., pp. 431-451, 1992.

53. Melzack, R. and Casey, K.L., Sensory, motivational and central control determinants of pain: A new conceptual model. In D. Kenshalo (Ed.), The skin senses. New York: Charles C. Thomas, pp. 423-439, 1968.

54. Mesches, M.H., Bianchin, M. and McGaugh, J.L. The effects of intra-amygdala infusion of the AMPA receptor antagonist CNQX on retention performance following aversive training. *Neurobiol. Learn. Mem.* 66: 324-40, 1996.

55. Millan, M.J. Serotonin and pain: evidence that activation of 5-HT1A receptors does not elicit antinociception against noxious thermal, mechanical and chemical stimuli in mice. *Pain* 58: 45-61, 1994.

56. Murphy, A.Z., Murphy, R.M. and Zemlan, F.P. Role of spinal serotonin1 receptor subtypes in thermally and mechanically elicited nociceptive reflexes. *Psychopharmacol.* 108: 123-30, 1992.

57. Oliveto, A.H. and Dykstra, L.A. Naloxone sensitivity in squirrel monkeys under a schedule of shock titration. *Pharmacol. Biochem. Behav.* 26: 191-3, 1987.

58. Paxinos, G. and Watson, C. The rat brain in stereotaxic coordinates, compact third edition. Academic Press, San Diego, 1997.

59. Phillips, R.G. and LeDoux, J.E. Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behav. Neurosci.* 106: 274-85, 1992.

60. Quillfeldt, J.A., Zanatta, M.S., Schmitz, P.K., Quevedo, J., Schaeffer, E., Lima, J.B., Medina, J.H. and Izquierdo, I. Different brain areas are involved in memory expression at different times from training. *Neurobiol. Learn. Mem.* 66: 97-101, 1996.

61. Rodgers, R.J. and File, S.E. Exploratory behaviour and aversive thresholds following intra-amygdaloid application of opiates in rats. *Pharmacol. Biochem. Behav.* 11: 505-11, 1979.

62. Romanski, L.M. and LeDoux, J.E. Equipotentiality of thalamo-amygdala and thalamo-cortico-amygdala circuits in auditory fear conditioning. *J. Neurosci.* 12: 4501-9, 1992.

63. Sananes, C.B. and Davis, M. N-methyl-D-aspartate lesions of the lateral and basolateral nuclei of the amygdala block fear-potentiated startle and shock sensitization of startle. *Behav. Neurosci.* 106:72-80, 1992.

64. Shi, C. and Davis, M. Pain pathways involved in fear conditioning measured with fear-potentiated startle: lesion studies. *J. Neurosci.* 19: 420-30, 1999.

65. Shi, C.J. and Cassell, M.D. Cascade projections from somatosensory cortex to the rat basolateral amygdala via the parietal insular cortex. *J. Comp. Neurol.* 399: 469-91, 1998.

66. Shi, C.J. and Cassell, M.D. Cortical, thalamic, and amygdaloid connections of the anterior and posterior insular cortices. *J. Comp. Neurol.* 399: 440-68, 1998.

67. Sipes, T.A. and Geyer, M.A. Multiple serotonin receptor subtypes modulate prepulse inhibition of the startle response in rats. *Neuropharmacology* 33: 441-8, 1994.

68. Thompson, R.F. and Spencer, W.A. Habituation: a model phenomenon for the study of neuronal substrates of behavior. *Psychol. Rev.* 73: 16-43, 1966.

69. Vaccarino, A.L. and Melzack, R. Analgesia produced by injection of lidocaine into the anterior cingulum bundle of the rat. *Pain* 39: 213-9, 1989.

70. Viaud, M.D. and White, N.M. Dissociation of visual and olfactory conditioning in the neostriatum of rats. *Behav. Brain Res.* 32: 31-42, 1989.

71. Vogt, B.A., Sikes, R.W. and Vogt, L.J., Anterior cingulate cortex and the medial pain system. In B.A. Vogt and Gabriel, M. (Eds.), Neurobiology of cingulate cortex and limbic thalamus: A comprehensive handbook. Boston: Birkhaeuser, pp. 313-344, 1993.

72. Walker, D.L. and Davis, M. Double dissociation between the involvement of the bed nucleus of the stria terminalis and the central nucleus of the amygdala in startle increases produced by conditioned versus unconditioned fear. *J. Neurosci.* 17: 9375-83, 1997.

73. Werka, T. The effects of the medial and cortical amygdala lesions on post-stress analgesia in rats. *Behav. Brain Res.* 86: 59-65, 1997.

74. Willis, W.D. and Westlund, K.N. Neuroanatomy of the pain system and of the pathways that modulate pain. *J. Clin. Neurophysiol.* 14: 2-31, 1997.