Psychopharmacology (1985) 87:278–282

Psychopharmacology

Fear-enhanced acoustic startle is not attenuated by acute or chronic imipramine treatment in rats

James V. Cassella and Michael Davis

Yale University School of Medicine and The Abraham Ribicoff Research Facilities of The Connecticut Mental Health Center, New Haven, CT 06508, USA

Abstract. The effect of acute or chronic administration of imipramine on fear-enhanced startle (potentiated startle) in rats was investigated. Thirty male albino rats were initially given preliminary startle testing, assigned to one of three matched groups, and trained for potentiated startle by presenting ten light-shock pairings on each of 2 days. Subsequent startle testing following a single injection of 0, 5 or 10 mg/kg imipramine revealed that the degree of startle potentiation (increased responding in the presence of the light previously paired with shock) was similar across treatment conditions. A significant and comparable potentiation of startle was observed in animals treated chronically with saline or imipramine (10 mg/kg/day) for 21 days between training and testing. Potentiated startle was also observed in these animals on the next (22 nd) day after injection of an additional dose of the drug (10 mg/kg) 5 min prior to testing. Plasma levels of imipramine and its metabolite, desipramine, were relatively high after each of these treatments. Since previous studies have shown that potentiated startle is decreased by diazepam, the present findings suggest that the potentiated startle paradigm is a valid model for studying simple fear or anxiety rather than panic disorder.

Key words: Anxiety – Fear – Imipramine – Panic disorder – Startle – Tricyclic antidepressants

Brown et al. (1951) originally demonstrated that the amplitude of the acoustic startle reflex in the rat could be increased if startle were elicited in the presence of a light previously paired with a shock. The increase in startle magnitude under these conditions has subsequently been termed the potentiated startle effect, and is believed to be mediated by conditioned fear (Brown et al. 1951). Pharmacological data support a fear interpretation, since potentiated startle is reduced by drugs such as sodium amytal (Chi 1965), diazepam (Berg and Davis 1984; Davis 1979a), and morphine (Davis 1979b) which reduce fear or anxiety in people. Conversely, potentiated startle is enhanced by drugs like yohimbine or piperoxane (Davis et al. 1979) which tend to increase anxiety in humans (e.g., Charney et al. 1982; Garfield et al. 1967; Holmberg and Gershon 1961; Soffer 1954). Therefore, the potentiated startle paradigm is a useful behavioral model for analyzing the neuropharmacological mechanisms underlying fear or anxiety.

Clinically, a severe form of chronic fear or anxiety is manifested as panic attacks. However, pharmacological data suggest that panic differs from non-panic forms of chronic anxiety, since these two disorders are treated by different classes of drugs. Panic attacks are effectively treated by antidepressants like imipramine (e.g., Klein and Fink 1962; Mavissakalian et al. 1984; Sheehan et al. 1980; Zitrin et al. 1983) but not by minor tranquilizers like diazepam (e.g., McNair and Kahn 1981; Sheehan 1982; Sweeney et al. 1983). Conversely, non-panic chronic anxiety is effectively treated by diazepam (e.g., Hallstrom et al. 1981; Tyrer and Lader 1974) while imipramine is generally not beneficial (e.g., Zitrin et al. 1983; Zitrin et al. 1981). Thus, these data suggest that different neurochemical mechanisms may mediate panic and non-panic forms of fear or anxiety.

It is not clear a priori whether the potentiated startle paradigm is a model for panic or non-panic forms of fear or anxiety. Given the therapeutic distinction between imipramine and diazepam, it was hypothesized that if the potentiated startle paradigm is a valid model for studying non-panic fear or anxiety, then imipramine and diazepam should produce differential results on the potentiated startle effect. Since it has already been demonstrated that diazepam blocks the potentiated startle effect (Berg and Davis 1984; Davis 1979a), the present experiment evaluated the effects of acute, as well as chronic, imipramine on potentiated startle. If potentiated startle is not blocked by imipramine, support would be provided for the idea that the potentiated startle paradigm is a valid model for fear or anxiety and not for panic disorder.

Materials and methods

Animals

Male albino Sprague-Dawley rats (Charles River Co.) weighing between 300 and 400 g were used. The rats were housed in group cages of five rats each and maintained on a 12 h:12 h light/dark schedule. Food and water were continuously available. All subjects were drug-naive at the start of the experiment.

Offprint requests to: J.V. Cassella, Dept. of Psychiatry, Yale Univ. School of Medicine, 34 Park Street, New Haven, CT 06508, USA

Apparatus

Potentiated startle training. Five identical boxes ($30 \times 25 \times 25$ cm) were used for potentiated startle training. The sides and top of each box were constructed of aluminum, while Plexiglas composed the front and back walls. Each floor consisted of 4.8 mm stainless steel bars spaced 19 mm apart. The boxes were located on two shelves within a 1×1 m ventilated, sound-attenuating chamber. The conditioned stimulus (CS) was produced by an 8 W fluorescent light bulb (100 μs rise time) located on the outside of the back wall of each training box. The unconditioned stimulus (US) was shock generated by five LeHigh Valley constant-current shockers (SGS-004) located outside the chamber. Shock intensity was measured with a 1 K resistor across a differential channel of an oscilloscope in series with a 100 K resistor connected between adjacent floor bars in each training box. Current was defined as the RMS voltage across the 1 K resistor where $\text{mA} = 0.707 \times 0.5 \times$ peak-to-peak voltage. According to this method the shock current was 0.6 mA.

Potentiated startle testing. The apparatus used to measure startle has been described previously (Weiss and Davis 1976). Briefly, five separate stabilimeters were used to record the amplitude of the startle response. Each stabilimeter consisted of an $8 \times 15 \times 15$ cm Plexiglas and wire mesh cage suspended between compression springs within a steel frame. An 8 W fluorescent bulb identical to that used for training was attached to the back of each cage. Cage movement resulted in displacement of an accelerometer where the resultant voltage was proportional to the velocity of displacement. Startle amplitude was defined as the maximum accelerometer voltage that occurred during the first 200 ms after the startle stimulus was delivered and was measured with a specially designed sample-and-hold circuit interfaced to a PDP-11 computer. The stabilimeters were housed in a dimly-lighted, ventilated, sound attenuating chamber. Each cage was located 10 cm from a high frequency speaker (Radio Shack Supertweeter). The startle stimulus was a 50-ms burst of white noise having a rise-decay time of 5 ms. Background white noise, provided by a white noise generator, was 55 dB. Sound level measurements were made within the cages using a General Radio Model 1551-C sound level meter (A-scale).

Procedure

Acute imipramine. Prior to investigating the effect of acute imipramine treatment on potentiated startle, 30 naive rats were placed in the startle cages and after 5 min presented with 30 startle stimuli, ten at each of three different intensities (85, 100, and 115 dB). The varying-intensity stimuli were presented in a balanced, irregular order across this test session. The rats subsequently were divided into three groups of ten rats each with each group having a similar mean startle amplitude based on these 30 stimuli.

One day following this matching procedure, the rats were trained for potentiated startle. The animals were placed in the training boxes and after 5 min were presented with ten light-shock pairings. The shock was delivered during the last 500 ms of the 3700-ms duration light at an average intertrial interval of 4 min (range 3–5 min). The ten conditioning trials were presented on 2 consecutive days, giving a total of 20 conditioning trials.

One day later the animals were tested for potentiated startle. Each rat was injected intraperitoneally (IP) with either saline, 5, or 10 mg/kg imipramine HCl (based on the weight of the salt), placed in the startle cage and after 5 min presented with 20 noise bursts at each of three different stimulus intensities (85, 100, and 115 dB). The interstimulus interval was 30 s. Half of the startle stimuli at each intensity were presented in darkness (Noise-Alone) while the other half of the startle stimuli were presented 3200 ms after the onset of the 3700-ms duration light (Light-Noise). The ten occurrences of each of the six different trial types (e.g., Light-Noise at 85 dB) were presented in a balanced, irregular order across the test session.

Chronic imipramine. To evaluate the effects of chronic imipramine on potentiated startle, another group of 20 naive rats was given preliminary startle testing, assigned to one of four matched groups, and trained for potentiated startle as described above. However, 3 days of potentiated startle training were given to maximize the possibility that the conditioning would still be present several weeks after training. On each of the next 21 days, the rats were injected IP in their home cage with either saline or 10 mg/kg/day imipramine (5 mg/kg at 9 A.M. and 5 mg/kg at 5 P.M.). On Day 22 all rats received the morning injection of saline or imipramine and were tested in the early afternoon, at least 4 h after drug injection. All animals received their normal night injection.

The effect of acute administration of imipramine in rats treated chronically with saline or imipramine was assessed in these same rats on the next day. The animals again received their normal morning injection and testing was begun early in the afternoon. Five minutes prior to testing half the saline-treated rats were injected with saline and half with imipramine (10 mg/kg). Similarly, half of the chronic imipramine rats were injected with saline and half with imipramine (10 mg/kg). Immediately after testing the rats were decapitated and exsanguinated. Plasma imipramine levels were determined by high performance liquid chromatography (HPLC) according to the method of Proelss et al. (1978).

Results

Effects of acute imipramine. Table 1 presents the mean amplitude startle response on the Noise-Alone and Light-Noise trials following acute injection of either saline, 5 or

Table 1. Effect of acute imipramine on potentiated startle

Stimulus condition	Drug treatment		
	Saline ($N=10$)	Imipramine 5 mg/kg ($N=10$)	Imipramine 10 mg/kg ($N=10$)
Noise-Alone	48.3	42.9	44.9
Light-Noise	77.7	63.6	68.4
*A**	29.4 ± 5.4	20.7 ± 5.9	23.5 ± 4.5

* Mean ± standard error of the mean

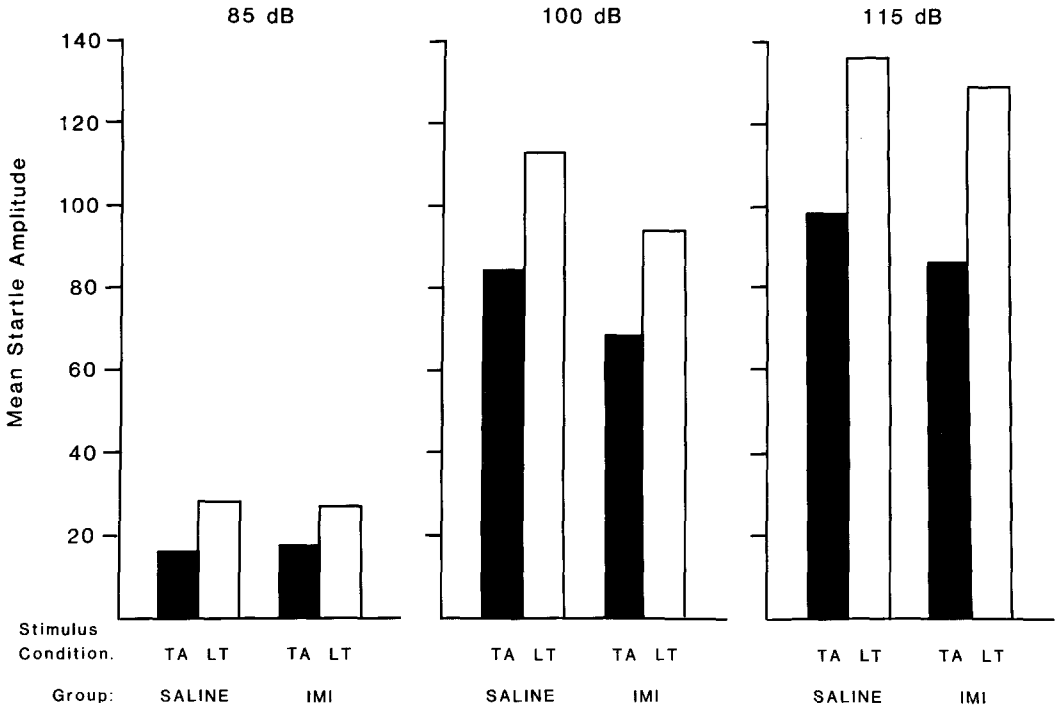

Fig. 1. Mean startle amplitude on Noise-Alone (*TA*) and Light-Noise (*LT*) trials at three stimulus intensities (85, 100, 115 dB) for animals treated chronically with saline ($N=10$) or imipramine ($N=10$; 10 mg/kg/day for 21 days)

10 mg/kg imipramine. Since analysis of variance (ANOVA) revealed a similar pattern of results across stimulus intensity [$F(4,54)=1.19$, $P>0.32$], the data were collapsed across these three test intensities. ANOVA conducted on the combined data yielded a significant main effect for Stimulus Condition [Noise-Alone versus Light-Noise; $F(1,27)=63.81$, $P<0.001$], indicating a robust potentiated startle effect. More importantly, the Treatment × Stimulus Condition interaction was not significant ($F<1$) suggesting that the degree of potentiation was comparable for all three treatment groups. The overall startle amplitudes were also similar across these treatment ($F<1$). These data can be compared to those reported previously by Davis (1979a), whereby a 1.25 or 2.50 mg/kg dose of diazepam essentially eliminated the potentiated startle effect without altering baseline startle levels.

Effects of chronic imipramine. Figure 1 displays the mean startle amplitude on the Noise-Alone (TA) and Light-Noise (LT) trials at each of the three stimulus intensities in rats treated chronically with either saline or imipramine (10 mg/kg/day). As the figure indicates, and ANOVA confirmed, startle amplitude was greater as the stimulus intensity increased, leading to a significant main effect for Intensity [$F(2,36)=74.67$, $P<0.001$]. Startle amplitude was significantly greater in the presence of the light, once again indicating a significant potentiated startle effect [$F(1,18)=29.92$, $P<0.001$]. In addition, the degree of potentiation was greater as the stimulus intensity increased [$F(2,36)=10.14$, $P<0.001$]. This potentiation is even more impressive considering that training occurred more than 3 weeks previously. Most importantly, the degree of potentiation was not different between animals treated chronically with either saline or imipramine as indicated by the non-significant Treatment × Stimulus Condition ($F<1$) and Treatment × Stimulus Condition × Intensity ($F<1$) interactions.

Table 2. Effect of acute imipramine in rats treated chronically with saline or imipramine

Chronic drug treatment		Acute drug treatment	
		Saline	Imipramine
Saline	TA	40.2	36.8
	LT	55.7	58.0
	Δ*	15.5±8.6	21.2±9.6
Imipramine	TA	31.1	35.9
	LT	43.3	63.9
	Δ	14.2±6.4	28.0±11.2

* Mean ± standard error of the mean

$N=5$ each treatment condition

Effects of chronic plus acute imipramine. Table 2 presents the mean startle amplitude, combined over intensities, on the Light-Noise (LT) and Noise-Alone (TA) trials after acute administration of either saline or imipramine in rats treated chronically with either saline or imipramine. ANOVA revealed a significant main effect for Stimulus Condition [Noise-Alone versus Light-Noise; $F(1,16)=17.54$, $P<0.001$] but no significant Treatment effect ($F<1$) nor Treatment × Stimulus Condition interaction ($F<1$). Thus, neither chronic nor chronic-plus-acute treatment with imipramine blocked the potentiated startle effect.

Table 3 shows the mean plasma levels of imipramine and its metabolite, desipramine, after acute or chronic treat-

Table 3. Plasma levels of imipramine and its metabolite desipramine

Drug regimen	(*N*)	Plasma levels* (ng/ml)	
		Imipramine	Desipramine
Acute IMI (10 mg/kg)	(10)	178 ± 23	154 ± 14
Chronic IMI (10 mg/kg/day)	(10)	38 ± 13	227 ± 44
Chronic + Acute IMI	(10)	157 ± 50	431 ± 59

* Mean ± standard error of the mean

ment with imipramine. The failure of chronic imipramine to block potentiated startle cannot be attributed to a lack of sufficient blood levels of imipramine, since these values are within or above the clinical range in humans (e.g., Mavissakalian et. al. 1984) and are consistent with drug levels observed in rats given a similar treatment regimen (Daniel et al. 1981; van Wijk et al. 1977). Indeed, the greatest percent potentiated startle was found in rats possessing the highest combined levels of imipramine and desipramine following chronic plus acute imipramine injection (Table 2).

Discussion

The present data indicate that neither acute nor chronic administration of imipramine blocked the potentiated startle effect. In contrast, previous data have shown that potentiated startle can be blocked by barbiturates (Chi 1965), benzodiazepines (Berg and Davis 1984; Davis 1979a), and opiates (Davis 1979b). To the extent that these latter drugs are more effective clinically in treating chronic non-panic anxiety, whereas imipramine seems to be more effective in treating panic disorder, it appears that the potentiated startle paradigm is a better model of non-panic anxiety than of panic disorder. Moreover, the fact that fear conditioning was still very potent 3 weeks following training suggests that the potentiated startle paradigm is useful for investigating the effects of other chronic manipulations on fear or anxiety mechanisms.

Acute and chronic treatment with imipramine are known to produce a variety of neurochemical changes in the rat brain. Acutely, imipramine increases the availability of monoamines in the synaptic cleft, presumably by blocking transmitter uptake (cf. Sugrue 1981). Chronic treatment with imipramine leads to the down-regulation of norepinephrine (NE) beta and alpha-2 receptors, 5-HT2 receptors, and dopamine receptors, while NE alpha-1 receptors are up-regulated (for review see Sugrue 1981). In addition, chronic treatment results in a down-regulation of NE-coupled adenylate cyclase (Sugrue 1981) as well as a decrease in dopamine (e.g., Leonard and Kafoe 1976) and NE (e.g., Nielsen and Braestrup 1977) turnover. Given the absence of any effect of acute or chronic imipramine treatment on the potentiated startle response, it might be suggested that this behavior does not correlate with the activity of those neurochemical systems altered by imipramine treatment. However, direct biochemical and electrophysiological analyses are required to rule out these systems in the mediation of the potentiated startle effect.

Acknowledgements. This research was supported by NSF Grant BNS-81-20476, NIMH Grant MH-25642, NINCDS Grant NS-18033, Research Scientist Development Award MH-00004 to M. Davis, and the State of Connecticut. J. Cassella is supported by a Biological Science Training Program Fellowship. Thanks are due to Alex Marek and Lee Schlesinger for their help in conducting the experiments and Dr. John Kehne for his critical review of the manuscript. Special thanks are extended to Dr. Peter Jatlow for determination of plasma imipramine and desipramine levels and the Clinical Centers Grant (MH-30929) which supported this aspect of the project.

References

Berg WK, Davis M (1984) Diazepam blocks fear-enhanced startle elicited electrically from the brainstem. Physiol Behav 32:333–336

Brown JS, Kalish HI, Farber IE (1951) Conditioned fear as revealed by magnitude of startle response to an auditory stimulus. J Exp Psychol 41:317–328

Charney DS, Heninger GR, Sternberg DE (1982) Assessment of alpha-2 adrenergic autoreceptor function in humans: Effects of oral yohimbine. Life Sci 30:2033–2041

Chi CC (1965) The effect of amobarbital sodium on conditioned fear as measured by the potentiated startle response in rats. Psychopharmacologia 7:115–122

Daniel W, Adamus A, Melzacka M, Szymura J, Vetulani J (1981) Cerebral pharmacokinetics of imipramine in rats after single and multiple dosages. Arch Pharmacol 317:209–213

Davis M (1979a) Diazepam and Flurazepam: Effects on conditioned fear as measured with the potentiated startle paradigm. Psychopharmacology 62:1–7

Davis M (1979b) Morphine and naloxone: Effects on conditioned fear as measured with the potentiated startle paradigm. Eur J Pharmacol 54:341–347

Davis M, Redmond DE, Baraban JM (1979) Noradrenergic agonists and antagonists: Effects on conditioned fear as measured by the potentiated startle paradigm. Psychopharmacology 65:111–118

Garfield SL, Gershon S, Sletten I, Sundland DM, Ballou S (1967) Chemically induced anxiety. Int J Neuropsychol 426–433

Hallstrom C, Treasaden I, Guy-Edwards J, Lader M (1981) Diazepam, propranolol and their combination in the management of chronic anxiety. Br J Psychiatry 139:417–421

Holmberg G, Gershon S (1961) Autonomic and psychic effect of yohimbine hydrochloride. Psychopharmacologia 2:93–106

Klein DF, Fink M (1962) Psychiatric reaction patterns to imipramine. Am J Psychiatry 119:432–438

Leonard BE, Kafoe WF (1976) A comparison of the acute and chronic effects of four antidepressant drugs on the turnover of serotonin, dopamine and noradrenaline in the rat brain. Biochem Pharmacol 25:1939–1942

Mavissakalian M, Perel JM, Michelson L (1984) The relationship of plasma imipramine and N-desmethylimipramine to improvement in agorophobia. J Cl Psychopharmacology 4:36–40

McNair DM, Kahn RJ (1981) Imipramine compared with a benzodiazepine for agorophobia. In: Klein DF, Rabkin J (eds) Anxiety: New research and changing concepts. Raven Press, New York, pp 69–79

Nielsen M, Braestrup C (1977) Chronic treatment with desipramine caused a sustained decrease of 3,4-dihydroxyphenylglycol-sulphate and total 3-methoxy-4-hydroxyphenylglycol in the rat brain. Arch Pharmacol 300:87–92

Proelss HF, Lohmann HL, Miles DG (1978) High-performance liquid – chromatographic simultaneous determination of commonly used tricyclic antidepressants. Clin Chem 24:1948–1953

Sheehan DV (1982) Panic attacks and phobias. N Engl J Med 307:156–158

Sheehan DV, Ballenger J, Jacobsen G (1980) Treatment of endogenous anxiety with phobic, hysterical, and hypochondriacal symptoms. Arch Gen Psychiatry 37:51–59

Soffer A (1954) Regitine and benodaine in the diagnosis of pheochromocytoma. Med Clin North Am 38:375–384

Sugrue MF (1981) Current concepts on the mechanisms of action of antidepressant drugs. Pharmacol Ther 13:219–247

Sweeney DR, Gold MS, Pottash ALC, Martin D (1983) Plasma levels of tricyclic antidepressants in panic disorder. Int J Psychiatr Med 13:93–96

Tyrer PJ, Lader MH (1974) Response to propranolol and diazepam in somatic and psychic anxiety. Br Med J 2:14–16

Van Wijk M, Meisch JJ, Korf J (1977) Metabolism of 5-hydroxytryptamine and levels of tricyclic antidepressant drugs in rat brain after acute and chronic treatment. Psychopharmacology 55:217–223

Weiss GT, Davis M (1976) Automated system for acquisition and reduction of startle response data. Pharmacol Biochem Behav 4:713–720

Zitrin CM, Klein DF, Woerner MG, Ross DC (1983) Treatment of phobias I. Comparison of imipramine hydrochloride and placebo. Arch Gen Psychiatry 40:125–138

Zitrin CM, Woerner MG, Klein DF (1981) Differentiation of panic anxiety from anticipatory anxiety and avoidance behavior. In: Klein DF, Rabkin J (eds) Anxiety: New research and changing concepts, Raven Press, New York, pp 27–46

Received December 19, 1984; Final version March 11, 1985