Pharmacology Biochemistry & Behavior, Vol. 15, pp. 533–536, 1981. Printed in the U.S.A.

Transfer of Pentylenetetrazol Sensitization to Amygdaloid Kindling

DONALD P. CAIN

Department of Psychology, University of Western Ontario
London, Ontario, Canada N6A 5C2

Received 16 March 1981

CAIN, D. P. *Transfer of pentylenetetrazol sensitization to amygdaloid kindling.* PHARMAC. BIOCHEM. BEHAV. **15**(4) 533–536, 1981.—Rats repeatedly injected with initially subconvulsive doses of pentylenetetrazol (PTZ) eventually became sensitized to the drug (displayed twitching and myoclonic jerks). Other rats injected with increased doses of PTZ displayed both sensitization and generalized convulsions. Both groups subsequently developed generalized amygdaloid kindled seizures significantly faster than control rats, but did not differ significantly from each other in their rate of kindling. These results indicate that substantial transfer facilitation of electrical kindling occurs whether sensitization alone, or sensitization together with convulsions are induced by injection of PTZ.

Kindling Seizures Pentylenetetrazol Sensitization Transfer

THE repeated administration of a variety of convulsant agents in initially subconvulsant amounts results in the gradual development of a permanent state of seizure susceptibility—the kindling effect. Recent findings indicate that the change in seizure susceptibility that occurs during kindling is not always limited to an increased susceptibility to the original kindling agent, but also can result in an increased susceptibility to other kindling agents. This transfer effect is demonstrated when subjects that are initially kindled with electrical stimulation of the amygdala or electroconvulsive shock are found to be significantly more susceptible to seizures induced by alcohol withdrawal or by injection of pentylenetetrazol (PTZ) [1, 7, 8].

Although pharmacological kindling and the subsequent transfer of kindling to alcohol-withdrawal-induced seizures have been demonstrated [4,8], these phenomena are not well understood. The repeated administration of carbachol or cocaine has been reported to result in subconvulsive behavioral sensitization or the development of generalized convulsions, but repeated administration of these substances generally has not resulted in transfer facilitation of electrical amygdaloid kindling [3, 10, 11, 14]. In the only study in which this transfer effect has been demonstrated, the authors concluded that the transfer facilitation was wholly attributable to the faster electrical kindling observed in the subjects that had actually convulsed during the drug treatment, and that the sensitized subjects did not kindle faster than the control subjects [3]. However, the results of this study do not unequivocally support the conclusion that generalized convulsions are necessary for transfer facilitation of electrical kindling, and that behavioral sensitization alone does not result in transfer. The exclusion of the convulsed subjects from the pharmacologically kindled group in order to assess the transfer effect that results from behavioral sensitization alone confounds response to the drug treatment with a change in the mean seizure susceptibility of the drugged group. This occurs because it might be expected that the more inherently seizure-prone subjects would convulse in response to the drug treatment, and if these subjects are then excluded, the drugged and control groups are no longer comparable. The same type of confound might be expected to occur in similar studies when subjects die during a convulsion induced by drug treatment (e.g. [10, 12, 13]). Therefore, the only useful comparison is between intact groups that have received administration of either a kindling agent or a control treatment and have different sensitization or seizure histories prior to transfer testing.

This approach was taken in the study reported here in an attempt to evaluate whether the induction of behavioral sensitization alone by the injection of PTZ is sufficient to result in transfer facilitation of electrical kindling, or whether generalized convulsions also must be evoked. The results indicate that substantial transfer occurs whether sensitization alone, or sensitization together with generalized convulsions are induced.

METHOD

Twenty-six male hooded rats weighing 350 to 400 g served as subjects. They were anesthetized with pentobarbital and received implantation of two bipolar electrodes using conventional stereotaxic techniques. Eighteen subjects carried an electrode in each basolateral amygdala, and the remaining eight subjects carried one electrode in the basolateral amygdala and one electrode in the contralateral dorsal hippocampus. These two sites were chosen to allow recording of neural activity from two major limbic regions during subsequent drug treatment. Electrodes were constructed of twisted Nichrome wire 127 μm in diameter, insulated except at the cut tips.

After a 14-day recovery period during which the subjects were handled periodically, they were randomly divided into

TABLE 1

BEHAVIORAL SENSITIZATION AND CONVULSIONS OBSERVED DURING DRUG TREATMENT

Group	N	Mean convulsive response to PTZ or saline after injection number: 1	25	Final	Number (percent) of subjects displaying a generalized convulsion during drug treatment	Mean number (range and SEM) of generalized convulsions displayed during drug treatment
Control	6	0	0	0	0 (0)	0 (no range)
Sensitized	11	0.36	2.27*	2.45	2 (18)	0.27 (0–2; ±0.19)
Convulsed	7	0.43	2.43†	3.0	7 (100)	2.30 (2–3; ± 0.18)

*Significantly greater than injection 1 ($p<0.005$).
†Significantly greater than injection 1 ($p<0.02$).

one control (n=7) and two experimental groups, the convulsed group (n=7) and the sensitized group (n=12). Subjects in the convulsed and sensitized groups were then weighed and received an intraperitoneal injection of PTZ (Sigma) at a dose of 20 mg/kg once every 48 hr until 25 injections had been administered. PTZ was used because it had been observed in our laboratory that repeated administration of this drug at a low dose reliably leads to sensitization without the risk of status epilepticus. The subjects were then placed on a large open surface and observed for 20 min. Occurrences of the following convulsive behaviors were recorded: (1) locomotor immobility with twitching of the facial musculature, (2) myoclonic jerks of the head and body, (3) forelimb clonus and convulsion generalization. The control subjects were treated similarly, but were injected with an equivalent volume of physiological saline instead of PTZ. Up to this point in the procedure, only two of the subjects in the convulsed group had displayed a generalized convulsion in response to the injections of PTZ. Therefore, in order to insure that all of the subjects in this group had displayed at least two generalized consulsions, all subsequent injections were administered at increased doses until a mean of 2.3 (range: 2–3) generalized convulsions were displayed. The dose of PTZ was increased by 5 mg/kg after every second injection. Thus, the convulsed subjects received two additional injections at 25 mg/kg, two additional injections at 30 mg/kg, etc., until 2–3 generalized convulsions were displayed. The maximum dose of PTZ injected was 35 mg/kg, and the subjects received a mean of 30.8 injections (range: 29–32). The subjects in the sensitized group received five additional injections at 20 mg/kg, for a total of 30 injections, and the control subjects received five additional injections of saline for a total of 30 injections.

Periodically throughout the experiment the EEG was monitored in each subject. A miniature plug and shielded leads connected the subject to a polygraph and permanent records of brain activity were taken for approximately 5 min before and 20 min after the injection of PTZ.

After a 7-day rest the subjects were electrically kindled through an amygdaloid electrode. The subjects were again connected to a polygraph and stimulator and the EEG was recorded before and after stimulation in order to obtain a prestimulation baseline and to record afterdischarge (AD). During the stimulation the leads to the polygraph were shorted to ground by a relay to prevent blocking of the amplifiers. For kindling, 1 sec of 60 Hz biphasic square waves, each 1 msec in duration, was provided by a constant current stimulator. The AD threshold was determined by setting the initial stimulation intensity at 40 μA and gradually raising the current until AD was observed. The following sequence of current values (in μA) was used: 40, 50, 75, 100, etc. Stimulations were spaced at 24 hr intervals. All subjects were then stimulated once daily at 200 μA, which was well above threshold for all subjects, until a generalized convulsion was displayed [9].

At the end of testing all subjects were anesthetized and perfused with formol saline, and the brain was removed, frozen, and sectioned for verification of the electrode placements. Two of the subjects, one each from the sensitized and control groups, had kindling-electrode placements outside the amygdala and were eliminated from the study. The remaining 24 subjects had electrode placements in the intended structures.

The results were analyzed using 2-tailed nonparametric tests of significance.

RESULTS

In order to quantify the degree of behavioral sensitization developed by the subjects during the drug treatment, the two behaviors indicative of sensitization (twitching and myoclonic jerks) were arbitrarily assigned a value of 1 and 2 respectively, and the mean sensitization response to the 1st, 25th, and final injection was calculated. The maximum score possible was 3.0, which indicated the occurrence of both twitching and myoclonic jerks. The values are presented in Table 1, where it can be seen that both experimental groups displayed little or no response to the 1st injection of PTZ, and a much stronger response to the 25th injection. The two experimental groups did not differ significantly in their response to the 1st or 25th injection (Mann-Whitney U, $p>0.05$). However, each experimental group displayed a significantly increased response to the 25th injection compared to the 1st injection (Sign test, convulsed group: $p<0.02$, sensitized group: $p<0.005$). The progressive increase in the convulsive response that occurred during the 25 injections of PTZ at 20 mg/kg was similar to that reported previously [5,8].

As was described above, all of the subjects in the convulsed group displayed between 2–3 generalized convulsions by the end of the drug treatment (Table 1). Within the sensitized group 2 of 11 subjects displayed 1 and 2 generalized convulsions each, respectively, by the end of the drug treat-

TABLE 2

ELECTRICAL KINDLING OF THE AMYGDALA IN CONTROL, SENSITIZED AND CONVULSED SUBJECTS

Group	Mean Number of ADs to 1st generalized seizure (range and SEM)	Median AD threshold in μA (range and SEM)
Control	13.2 (10–17; ± 1.0)	40.0 (40–100; ± 12.2)
Sensitized	9.5* (7–15; ± 0.7)	40.0 (40– 75; ± 4.8)
Convulsed	7.6† (6– 8; ± 0.3)	40.0 (40–100; ± 8.0)

*Significantly less than Control ($p<0.02$).
†Significantly less than Control ($p<0.01$).

ment. The remaining 9 sensitized subjects did not display any generalized convulsions during the drug treatment.

The recordings obtained from the amygdala and hippocampus during the course of the drug treatment indicated that low- or moderate-amplitude sharp waves occurred in these structures coincident with twitching induced by injection of PTZ, and that single high-amplitude spikes occurred coincident with myoclonic jerks in all recorded cases. There was never evidence of sustained trains of epileptiform spikes or other AD-like phenomena during twitching or myoclonic jerks. During generalized convulsions induced by injection of PTZ in the convulsed group a sustained train of high-amplitude spikes that was very reminiscent of evoked AD invariably occurred coincident with the convulsion. The electrographic and behavioral generalized seizure generally lasted between 30–50 sec and was followed by isolated myoclonic jerks and associated high-amplitude spikes in the EEG.

The main results of the study are presented in Table 2, where it can be seen that the sensitized group required fewer ADs to kindle than the control group, and the convulsed group required fewer ADs than the sensitized group. Because of heterogeneity of variances these data were analyzed using nonparametric ANOVA, which indicated that the groups differed significantly (Kruskall-Wallis, $p<0.01$). The results of individual comparisons indicated that the sensitized and convulsed groups kindled significantly faster than the control group (Mann-Whitney U, $p<0.02$ and $p<0.01$ respectively), but that the sensitized and convulsed groups did not differ significantly ($p\geqslant 0.05$). The median initial AD thresholds (Table 2) did not differ significantly ($p\geqslant 0.05$).

In order to assess whether the total of three generalized convulsions displayed in response to the PTZ injections by two subjects in the sensitized group contributed to the transfer observed in this group, the data relating to the two subjects in question were eliminated, and a second analysis of the results was performed. Eliminating the two subjects from the sensitized group changed the value of the mean number of ADs required for electrical kindling from 9.5 to 9.8. This value was also significantly less than that of the control group (Mann-Whitney U, $p<0.05$), but did not differ significantly from that of the convulsed group (Mann-Whitney U, $p\geqslant 0.05$).

DISCUSSION

The major requirements for a test of transfer as outlined in the Introduction appear to have been met in this study. Subjects were randomly assigned to groups that received different drug treatments and therefore had different seizure histories at the beginning of electrical kindling. The two subjects that initially were eliminated from the study were eliminated solely on the grounds of inadequate electrode placements, and could not have systematically biased the mean seizure susceptibility of the groups. The two experimental groups did not differ in their initial response or in the degree of behavioral sensitization displayed after 25 injections of PTZ at 20 mg/kg, indicating that they were comparable in both baseline seizure susceptibility and in sensitization responsivity to repeated administration of PTZ. Both experimental groups displayed a significant increase in responsivity to PTZ during the initial series of 25 injections. Finally, 9 of 11 sensitized subjects displayed only sensitization by the end of the drug treatment, and all 7 convulsed subjects displayed both sensitization and convulsions.

Under the conditions of this study, sensitization due to the repeated injection of low doses of PTZ resulted in significant transfer facilitation of amygdaloid kindling. Increasing the dose of PTZ in order to induce 2–3 generalized convulsions in addition to the sensitization did not result in significantly greater transfer.

The term "sensitization" has been used widely in published reports to refer to both behavioral (e.g., behavioral stereotypy) and epileptic phenomena (e.g., lowered convulsive threshold, increased convulsion severity, etc). In the present report the term refers to the increase in ungeneralized seizure manifestations resulting from the repeated injection of PTZ.

The question whether the total of three generalized convulsions displayed by two subjects in the sensitized group contributed in any substantial way to the transfer observed in this group cannot be answered by eliminating them from the group without confounding the study, as was indicated earlier. However, for the same reason, eliminating these two subjects from the sensitized group, might be expected to reduce the mean seizure susceptibility of the sensitized group, and therefore make it less likely that the remaining nine subjects in this group would be statistically distinguishable from the control group in their rate of electrical kindling. The fact that the results of the second, more conservative, analysis were essentially identical to those of the first analysis performed on the complete data supports the conclusion that, under the conditions of this experiment, the greatest increment in transfer results from sensitization by PTZ, and that a small number of generalized convulsions adds little to the effect. It is possible, of course, that additional generalized convulsions induced by additional injections of PTZ might facilitate electrical amygdaloid kindling further.

The possibility that spontaneous convulsions might have occurred during the interinjection interval and contributed to the transfer effect seems unlikely for a number of reasons. Although no systematic long-term recordings were taken during the interinjection period, the subjects were under intermittent observation during this period and no spontaneous convulsions were observed in any subject in this study. In our experience and in the experience of others the hooded rat is very resistant to spontaneous seizures produced by kindling treatment, and spontaneous seizures typically develop only after many hundreds of generalized seizures have been evoked by high-frequency electrical stimulation [2,6].

The median AD threshold of the groups did not differ. Although 18 of the 24 subjects kindled in this study displayed

AD in response to the initial stimulation of 40 μA during AD threshold testing, the mean AD threshold of the groups was not identical due to small variations in the AD threshold of a few subjects in each group at the high end of the AD-threshold range (see Table 2). Stimulations of 200 μA intensity were applied to all subjects in order to provide stimulation that was of equal intensity for all subjects, and that was at least twice the AD threshold of all subjects. The results of previous research have indicated that such suprathreshold stimulation should be effective for the kindling of seizures at about the same rate, on average, in naive subjects [9].

The mechanism by which the sensitization induced by injection of PTZ facilitates amygdaloid kindling is unknown. PTZ appears to exert its convulsant effects by a variety of mechanisms including the antagonism of GABA-mediated inhibition, an increase in potassium permeability of neuronal membrane, the inhibition of acetylcholinesterase, and a direct excitatory effect [15]. Any or all of these mechanisms might induce changes in the neural substrate involved in electrical kindling of the amygdala, resulting in transfer facilitation. The EEG recordings taken after the injection of PTZ indicate that sharp waves, high-amplitude spikes, or sustained trains of spikes accompanied the convulsions, but do not prove that such EEG phenomena are necessary for transfer.

Whatever neural mechanism is involved in the transfer effect between injection of PTZ and electrical kindling of the amygdala, it should be noted that the transfer effect is bidirectional: electrical kindling of the amygdala significantly facilitates the response to PTZ [1,7], and, as has been demonstrated here, injection of PTZ significantly facilitates electrical kindling of the amygdala. Certain other pairs of convulsant agents have been found to exhibit unidirectional transfer in studies that employed a unidirectional test of transfer [8]. Whether these and other pairs exhibit bidirectional transfer effects is a question for further research.

REFERENCES

1. Cain, D. P. Effects of kindling or brain stimulation on pentylenetetrazol-induced convulsion susceptibility. *Epilepsia* **21**: 243–249, 1980.
2. Cain, D. P. and M. E. Corcoran. Kindling with low-frequency stimulation: Generality, transfer and recruiting effects. *Expl Neurol.*, in press, 1981.
3. Kilbey, M. M., E. H. Ellinwood and M. E. Easler. The effects of chronic cocaine pretreatment on kindled seizures and behavioral stereotypes. *Expl Neurol.* **64**: 306–314, 1979.
4. Mason, C. R. and R. M. Cooper. A permanent change in convulsive threshold in normal and brain-damaged rats with repeated small doses of pentylenetetrazol. *Epilepsia* **13**: 663–674, 1972.
5. Pinel, J. P. J. and K. F. Cheung. Controlled demonstration of metrazol kindling. *Pharmac. Biochem. Behav.* **6**: 599–600, 1977.
6. Pinel, J. P. J. and L. I. Rovner. Experimental epileptogenesis: Kindling-induced epilepsy in rats. *Expl Neurol.* **58**: 190–202, 1978.
7. Pinel, J. P. J., R. Skelton and R. F. Mucha. Kindling-related changes in afterdischarge "thresholds." *Epilepsia* **17**: 197–205, 1976.
8. Pinel, J. P. J. and P. H. Van Oot. Generality of the kindling phenomenon: Some clinical implications. In: *Kindling*, edited by J. A. Wada. New York: Raven Press, 1976, pp. 155–171.
9. Racine, R. J. Modification of seizure activity by electrical stimulation: II. Motor seizure. *Electroenceph. clin. Neurophysiol.* **32**: 281–294, 1972.
10. Rackham, A. and R. A. Wise. Independence of cocaine sensitization and amygdaloid kindling in the rat. *Physiol. Behav.* **22**: 631–633, 1979.
11. Sato, M., N. Hikasa and S. Otsuki. Experimental epilepsy, psychosis, and dopamine receptor sensitivity. *Biol. Psychiat.* **14**: 537–540, 1979.
12. Stripling, J. S. and E. H. Ellinwood, Jr. Augmentation of the behavioral and electrophysiologic response to cocaine by chronic administration in the rat. *Expl Neurol.* **54**: 546–564, 1977.
13. Stripling, J. S. and E. H. Ellinwood, Jr. Potentiation of the behavioral and convulsant effects of cocaine by chronic administration in the rat. *Pharmac. Biochem. Behav.* **6**: 571–579, 1977.
14. Wasterlain, C. G. and V. Jonec. Neurotransmitter interactions in chemical kindling. Paper presented at the meeting of the Society for Neuroscience, Cincinnati, OH, November 1980.
15. Woodbury, D. M. Convulsant drugs: Mechanisms of action. In: *Antiepileptic Drugs: Mechanisms of Action*, edited by G. H. Glaser, J. K. Penry and D. M. Woodbury. New York: Raven Press, 1980, pp. 249–303.