Pharmacology Biochemistry & Behavior, Vol. 22, pp. 435–439, 1985. © Ankho International Inc. Printed in the U.S.A. 0091-3057/85 $3.00 + .00

Dose-Related Effects of Pentobarbital on the Genetic Differences Seen Between Paired, Roman High- or Low-Avoidance Rats in a Shuttle Box[1]

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Received 21 February 1984

DRISCOLL, P. AND R. STÜBI. *Dose-related effects of pentobarbital on the genetic differences seen between paired, Roman high- or low-avoidance rats in a shuttle box.* PHARMACOL BIOCHEM BEHAV **22**(3) 435–439, 1985.—The effects of low doses of pentobarbital (PB) were measured on the activity levels, shock-induced fighting and avoidance or escape behavior of paired rats of two psychogenetically-selected lines, in multiple shuttle box sessions, following shock-induced fighting or two-way avoidance training. Each pair served as its own control, by receiving drug injections only every second week. Independent of training conditions, the RLA/Verh pairs showed about 90% freezing behavior and no fighting, whereas all RHA/Verh pairs preferred avoidance or escape behavior to fighting. Although their intertrial (shuttling) responses (ITRs) were reduced at the higher doses of PB used, RHA/Verh rats were still capable of most behavioral responses even at 24 mg/kg, whereas all RLA/Verh rats slept at that dose. On the other hand, the ITRs and avoidance responses of the (less active) RLA/Verh rats were increased by injections of 8 and 16 mg/kg PB. The results, especially those pertaining to freezing behavior and changes in activity levels, were discussed in comparison to other selected rat strains which have shown certain similarities to the Roman lines in regard to "emotionality" and associated neurochemical status.

Two-way avoidance Shock-induced fighting Pentobarbital Psychogenetics
Roman high- and low-avoidance rats Freezing behavior Pharmacogenetics

ROMAN high-avoidance (RHA/Verh) rats and Roman low-avoidance (RLA/Verh) rats have been bred, respectively, for rapid vs. non-acquisition of two-way avoidance [11]. Whereas RHA/Verh rats are comparable to most other stocks in regard to shock-induced fighting performance [10], RLA/Verh rats tend to freeze (remain immobile) in all shock-stress situations, regardless of whether they are alone or paired [13]. In addition to differences in activity levels [3], a particularly interesting aspect of this behavioral polarization, based on many additional behavioral and physiological comparisons, is that it appears primarily as though emotional differences underlie the divergent shock-motivated responses of the two rat lines [11].

It has often been maintained that shock-induced fighting is not a true form of aggression, but rather defensive in nature [6,28], or a means of coping with a stressful situation when no alternative response is available [32,34]. It would therefore be assumed that, if given the chance, an animal would prefer to avoid or escape shock in such a situation. Several studies, utilizing lever pressing [2], an escape runway [37] or changes in position and orientation [19,29], have indicated this to be the case. The present report deals, in part, with the question of how paired RHA/Verh or RLA/Verh rats react in a shuttle box (two-way avoidance) situation in which they have the chance to escape or avoid shock, rather than fight.

By preceding the experimental sessions with two sessions of shock-induced fighting training for half of the pairs, and two sessions of (individual) two-way avoidance training for the other half, the following comparisons were made possible: pairs of rats pre-trained to fight in response to, or to avoid, shock (RHA/Verh rats), or pairs of rats pre-exposed to shock-induced freezing (essentially an "inescapable shock" paradigm) either alone, or in a paired situation (RLA/Verh rats).

Behavioral differences between the two lines of rats in sensitivity to pentobarbital sodium (PB) have also been investigated in the present report. A previous study has shown that RLA/Verh rats are more sensitive to the toxic effects of

[1]This study was conducted at the Swiss Federal Institute of Technology and was partly supported by a grant from the Swiss Association of Cigarette Manufacturers.

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PB [9]. Although some investigators have found that low doses of barbiturates may potentiate shock-induced fighting (e.g. [8,18]), increase the tolerance of electric shock in order to obtain food or water rewards [15,34], or "reduce anxiety" in a choice-discrimination task [17], it has also been shown that PB, even at low doses, can increase avoidance or escape failures [21]. The present study, therefore, investigated the effects of graduated, low doses of PB on activity levels and shock-induced fighting, with possibilities for escape or avoidance, in the context of the genetic and situational factors mentioned above.

METHOD

Sixty, naive, male RHA/Verh and 60, naive, male RLA/Verh rats, all 6 months old, were used. These lines had been (out)bred for 40 generations at the time of the present study, first in Rome [5] and then in Birmingham, England [7] from which laboratory the present stocks were obtained in 1972. The rats were group-housed with food and water ad lib. All testing was done between 10.00 and 16.30 hr, during the lighted part of the 06.15–18.15 hr cycle. After one week of handling, each line was divided into 15 pairs, to be trained under shock-induced fighting conditions, and 30 rats to be trained singly under two-way, active avoidance conditions. All training and experimental sessions were conducted exactly one week apart for each subject, or pair. The apparatus used has been previously described in detail [11]. It consisted of a shuttle box with two 27×27×27 compartments, either of which could also be used for the shock-induced fighting training by replacing the door partition with a solid partition.

The training sessions took place during the second and third weeks, with the second of these sessions being preceded by an IP injection of physiological saline solution (NaCl-2 ml/kg) 1 hr before training, for each rat. In order to maintain a degree of consistency among the avoidance training, fight training and later experimental sessions, the following criteria were used for all sessions: 15 runs per session, a five sec CS (light)-US (onset of shock) interval, a shock level of 1 mA, an intertrial interval (ITI) of 30 sec, and a maximum shock duration of 10 sec (shorter, of course, following an avoidance or escape response).

The fourth through ninth weeks comprised the experimental sessions. The 30, singly-trained rats of each line were formed into five groups of three pairs each, as were the 15 pairs of each line which were previously trained under shock-induced fighting conditions, with care being taken to divide the pairs as equally as possible, based on performance, among the five groups. Each pair was its own control during the subsequent testing sessions. That is, each of the five groups of six avoidance-trained and six fight-trained pairs, equally divided between RHA/Verh or RLA/Verh, was injected with either 0 (NaCl), 4, 8, 16 or 24 mg/kg PB, IP, one hour before testing during the even-numbered weeks (nrs 4, 6 and 8). During the odd-numbered weeks (nrs 5, 7 and 9) all pairs received NaCl ("0" dosage) injections only. In other words, the dosage levels for one group (12 pairs) during weeks 4 through 9 were, respectively, 0, 0, 0, 0, 0, 0; for the second group (12 pairs) 4, 0, 4, 0, 4, 0; for the third group (12 pairs) 8, 0, 8, 0, 8, 0; etc.

During all experimental sessions, the normal shuttle box situation was adhered to, as had been used during the avoidance-training sessions, and the behavior of both members of the pairs was observed and recorded, using a number system. The various code numbers were subsequently combined into seven broad categories. These categories were freezing (or remaining motionless), escape, avoidance, running around (without interacting with the other rat), posturing/fighting, false entries or returns, and intertrial responses (ITRs). All of the dosage groups within each strain vs. training vs. behavior category were compared using one-way ANOVAs, followed by a modified Newman-Keuls test [32], which compared the drug results with their respective baseline (control) values.

RESULTS

By the conclusion of the second training session, the RHA/Verh pairs had achieved baseline levels of fighting and posturing comparable to levels previously reported, and the RLA/Verh pairs showed their usual pattern of freezing behavior [13]. Likewise, the single RHA/Verh rats had achieved an 80%, or higher, rate of avoidance, whereas the single RLA/Verh rats also consistantly froze upon presentation of the CS and US.

At higher dosage levels of PB during the experimental sessions (16 and 24 mg/kg), it can be seen, in Fig. 1 and Table 1, that RHA/Verh pairs remained motionless more often and avoided less than during their control sessions. They were still capable of escaping and/or avoiding even at 24 mg/kg, however, whereas all RLA/Verh rats slept at that dose. Independent of training conditions, the RLA/Verh pairs (which have all been combined) showed about 90% freezing and no posturing or fighting, whereas all RHA/Verh pairs preferred avoidance or escape behavior to posturing/fighting, the latter of which averaged only about 2–3% for most conditions. The values for posturing/fighting and "running around" have been omitted, as these behaviors were not frequently seen during weeks 4 through 9. Some tendencies were noted in the running around category, however, as follows: RHA/Verh about 3–8% for all groups, except for increases to 10–14% after higher doses (16 and 24 mg/kg) PB, and RLA/Verh about 3–4% for all groups, except for increases to 7–11% following injections of 8 and 16 mg/kg PB. It should be noted that during the experimental (PB injection) sessions, the ITRs of both avoidance- and fight-trained RHA/Verh pairs peaked at the dosage level of 4 mg/kg, corresponding with the avoidance frequencies seen during the experimental sessions after both types of training (Fig. 1). The same was true for the RLA/Verh rats at the dosage levels of 8 and 16 mg/kg.

DISCUSSION

In comparison to RHA/Verh pairs, RLA/Verh pairs showed predominantly freezing behavior, almost no posturing/fighting, and very little escape or avoidance behavior in the present study, during both the experimental and training sessions. These observations confirmed previous results with these and with other Roman lines in regard to shock-induced fighting [13,14] and with these lines in regard to shuttle box performance [11]. This behavior of RLA/Verh rats, for which they are selectively bred, is apparently dependent upon increased "anxiety" levels [11]. In this regard, it should be noted that the "more emotional" Maudsley Reactive (MR) rat strain has long been known to respond significantly poorer in escape-avoidance conditioning than has the "less-emotional" Maudsley non-reactive (MNR) rat strain [20], and it has also been recently demonstrated that MR rats show much less shock-induced fighting than do MNR rats [14].

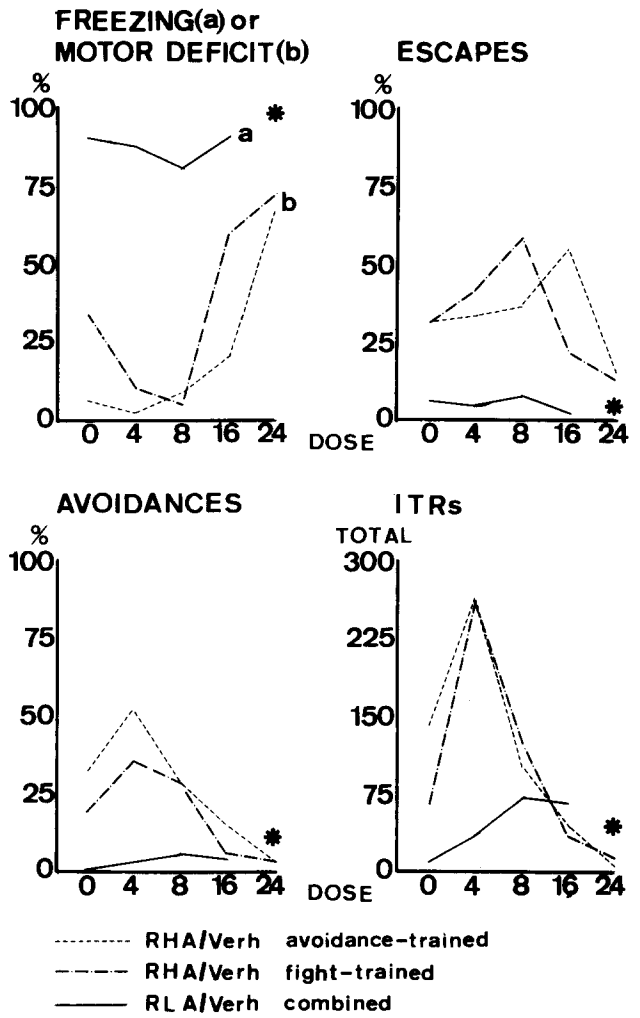

FIG. 1. Visual representation of most-frequently observed behaviors, over the PB dose range. Actual statistical differences, based on differences from baseline values, are presented in the Table. *=All RLA/Verh rats slept at that dose.

Irrespective of PB dose, it was seen that RHA/Verh pairs preferred avoidance and escape responses to fighting responses when given a choice, recalling similar findings of previous studies [2, 19, 29, 37]. In addition, the fact that both avoidance- and fight-trained RHA/Verh rats avoided and/or escaped about equally as well as each other when untreated, or even at the lower PB doses, would appear to indicate that prior, signalled shock exposure (without restraint) had a facilitative effect upon subsequent avoidance behavior, even if the shock was "inescapable" [1]. The question remains, however, whether shock-induced fighting training in a sealed enclosure should be regarded as a form of inescapable shock, or whether it provides a means of coping with (i.e., actively responding to) a stressful situation, as has been suggested previously [33,35].

Whereas, at the highest dose of PB (24 mg/kg), the RHA/Verh pairs still showed a considerable level of escape behavior and even some avoidance behavior, despite a sharp decrease in activity which was probably due to a sedative or motor deficit effect of PB, all RLA/Verh pairs slept at the time of testing at that dose and could not be tested in the

TABLE 1

DIFFERENCES FROM THE RESPECTIVE CONTROL BASELINES FOR THE MOST-FREQUENTLY OBSERVED BEHAVIORS, OVER THE PB DOSE RANGE

	No Movement					Escapes					Avoidances					ITRs				
Dose	0	4	8	16	24	0	4	8	16	24	0	4	8	16	24	0	4	8	16	24
RHA/Verh avoidance-trained	+11 D=65	+1	+2	+37	+133*	+20 D=47	+4	−9	+62*	−54*	−16 D=70	−22	−11	−80*	−96*	−19 D=170	+17	−73	−184*	−248*
RHA/Verh fight-trained	+6 D=66	−18	0	+70*	+118*	+17 D=61	+18	+53	−13	−94*	−14 D=59	+6	−59	−53	−55	−31 D=158	+72	−180*	−112	−109
RLA/Verh combined	+1 D=14	+9	+3	+24*	slept	+4 D=13	−10	−8	−11	slept	−1 D= 6	0	+8*	+7*	slept	0 D =34	+20	+33	+37*	slept

*$p<0.05$.

shuttle box. This result was not unexpected, as RLA/Verh rats have been shown to be more sensitive than RHA/Verh rats to the toxic effects of PB and there is evidence for differences in liver metabolism between the two lines [9]. Differences have also been found in the debilitating effects of other, liver-metabolized, substances as well, such as phenylbutazone [12] and oxotremorine [23], and it is also likely that RLA/Verh rats are less tolerant than are RHA/Verh rats to the effects of alcohol [22]. In this regard, it has been shown that "most affected" rats, which have been bred for increased sensitivity to alcohol, are more likely to lose their righting reflex and sleep longer than "least affected" rats after being injected IP with 18 mg/kg PB [26].

Based on the measurement of ITR's and avoidance responses, it was seen that PB disinhibited RLA/Verh rats at doses of 8 and 16 mg/kg. These increases in activity were not accompanied by increased fighting (e.g. [8,18]), but by slight increases in running around. The reduction in freezing behavior which permitted all of these changes may have reflected a slight drop in the level of anxiety, which has been attributed previously by some to comparable doses of PB in various tests with rats [15, 17, 34]. In that regard, it should be noted that the "less anxious" and normally more active RHA/Verh rats had generally fewer ITRs than did their respective controls at those dosage levels. Although they did show a comparable increase in running around, this was not accompanied by a reduction in freezing behavior, but rather by a reduction in avoidances, indicating (two-fold) that PB was already exerting a sedative or motor deficit effect at these doses [21], which was essentially the opposite result as that found with the RLA/Verh rats.

Both PB and other "anti-anxiety" drugs are presently being tested for possible effects during the training and maintenance stages of two-way avoidance in the "more anxious" RLA/Verh line, in order to form more definite conclusions regarding the potential, pharmacological alleviation of their stress-provoked fear responses. Recent studies have also shown, for example, that the benzodiazepine drug, chlordiazepoxide, increased entry into an illuminated open arena by RLA/Verh rats, whose normal baseline level of entry into the arena, which was located in the center of an unlighted maze, was much lower than that of RHA/Verh rats [4,24]. The point has been previously made here that certain similarities in avoidance and shock-induced fighting behavior exist between RLA/Verh and MR rats, as compared to RHA/Verh and MNR rats, respectively [11, 13, 14, 20]. Comparable similarities in benzodiazepine receptor binding also exist, as both the "more emotional" MR strain [27] and RLA/Verh line [16] show lower specific ^{3}H-diazepam binding than do their respective counterparts, in several of the same brain regions. These findings are of interest in that both drugs are widely believed to exert many of their pharmacological effects by influencing one or more of the same receptor sites [25, 30, 31, 36].

ACKNOWLEDGEMENTS

The authors wish to thank Professor K. Bättig and Dr. P. Woodson for their advice and support, and Mrs. C. Vaclavik for assistance in preparing the manuscript.

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