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# Suppression of pentobarbitone-induced hyperactivity by past experience in mice

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**Abstract**—Locomotor activity of CD-1 mice, tested in an unfamiliar environment (toggle-floor box), was increased either by a subhypnotic dose (20 mg kg$^{-1}$) of pentobarbitone or after recovery from pentobarbitone-induced (50 mg kg$^{-1}$) anaesthesia. On the contrary, when mice were tested 6 h after a single exposure to the apparatus, pentobarbitone in either case failed to produce hyperactivity. The results demonstrate that mice recovering from barbiturate anaesthesia maintain susceptibility to the exteroceptive stimuli provided by a novel environment and knowledge of the environment acquired during past experience.

Although generally regarded as sedatives, under some conditions, barbiturates may stimulate locomotor activity in laboratory animals (Stretch 1963; Harris et al 1966; Watzman et al 1968). Excitement induced by barbiturates is particularly interesting from the practical point of view as it is regarded as one of the unwanted but not uncommon side-effects of the drugs given as hypnotics (Harvey 1975). Moreover, such an excitement can be sometimes observed during recovery from barbiturate anaesthesia (Price 1975). We have recently observed that hyperactivity occurs not only in mice receiving subhypnotic doses of pentobarbitone (pentobarbital), but also in animals recovering from pentobarbitone-induced anaesthesia (Vetulani et al 1989). It is not known if the mechanism of these two kinds of hyperactivity is the same. The barbiturate-induced locomotor stimulation occurs when brain concentration of the drug approaches a particular level (Middaugh et al 1981) and this level can be reached after the administration of a suitable subhypnotic dose as well as during recovery from anaesthesia, when brain concentration of the barbiturate declines. Thus, it is likely that the same neurochemical mechanisms are responsible for both types of pentobarbitone-induced hyperactivity, but it cannot be excluded that the two barbiturate behavioural features might be differently influenced by the presence or absence of barbiturate metabolites as well as by environment and social factors.

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Since novelty of the environment is an important factor in the locomotor stimulatory action of drugs (Steinberg et al 1961; Rushton et al 1963; Oliverio & Castellano 1974), in the present study we investigated how a previous experience—the knowledge of an apparatus used for measurement of locomotor activity acquired during a single test carried out on naive, non drugged mice—affects the stimulatory action of a subhypnotic dose of pentobarbitone and posthypnotic hyperactivity.

## Materials and methods

The subjects were naive male mice (27–32 g) of the randomly bred CD-1 strain (Charles River, Italy). Upon their arrival in the laboratory (7–10 days before the experiment), the mice were housed in standard transparent plastic cages (8 per cage), under standard laboratory conditions (free access to food and water, ambient temperature of 22°C, light on from 7 am to 7 pm). 48 h before the experiment, mice were placed in single 30 × 12 × 12 cm transparent plastic cages, where they remained till the test for locomotor activity.

The locomotor activity was measured in an apparatus consisting of 8 toggle-floor boxes, each divided into two 20 × 10 cm compartments connected by a 3 × 3 cm opening. For each mouse, the number of crossings from one compartment to the other was automatically recorded by means of a microswitch connected to the tilting floor of the box. The apparatus was located in a sound-insulated cubicle.

Inexperienced animals had never been in the toggle-floor box before the activity test. Experienced mice were placed for 30 min in the toggle-floor box, without treatment, 6 h before the activity test (this interval slightly varied in the case of the animals receiving the sleep-inducing dose of barbiturate). The treatment with a subhypnotic dose of pentobarbitone sodium (Clin-Midi, France; 20 mg kg$^{-1}$) took place 15 min before a 30 min activity test; the controls received saline solution (0·9% NaCl). Posthypnotic activity was measured, during 30 min, starting 15 min after the recovery of the righting reflex, in mice receiving 50 mg kg$^{-1}$ pentobarbitone sodium, a dose which produced sleep lasting

between 30 and 60 min (42 min on average); the controls received saline solution, 60 min before testing. All injections were made intraperitoneally in a volume of 10 mL $kg^{-1}$.

## Results and discussion

The mean activity crossings, exhibited by the mice during the 30-min test, are reported in Fig. 1 for all the experimental groups. A 2 × 2 factorial ANOVA, concerning inexperienced and experienced mice receiving the subhypnotic dose of pentobarbitone and the respective controls, gave significant experience ($F(1,28) = 23{\cdot}82$, $P < 0{\cdot}001$) and pentobarbitone ($F(1,28) = 13{\cdot}69$, $P < 0.001$) main effects and a significant experience × barbiturate interaction ($F(1,28) = 4{\cdot}69$, $P < 0{\cdot}05$). Such interaction arose because pentobarbitone significantly increased the mean number of activity crossings in inexperienced, but not in experienced mice (Fig. 1). In the same way, significant main effects of experience ($F(1,28) = 35{\cdot}69$, $P < 0{\cdot}001$) and pentobarbitone ($F(1,28) = 14{\cdot}15$, $P < 0{\cdot}001$) and a significant two-factor interaction ($F(1,28) = 15{\cdot}05$, $P < 0{\cdot}001$) were found, when the statistical analysis concerned posthypnotic activity. Also in this case, pentobarbitone-withdrawal hyperactivity occurred in inexperienced, but not in experienced mice (Fig. 1).

On the whole, the results demonstrate that novelty of the environment is crucial for the locomotor stimulation induced by a subhypnotic dose of pentobarbitone as well as for the hyperactivity exhibited by mice during recovery from pentobarbitone anaesthesia. Similar results, that is suppression of both types of barbiturate-induced hyperactivity by prior experience of the test apparatus, were obtained when mice were subjected to the activity test 24 h after a single environment experience (data not shown).

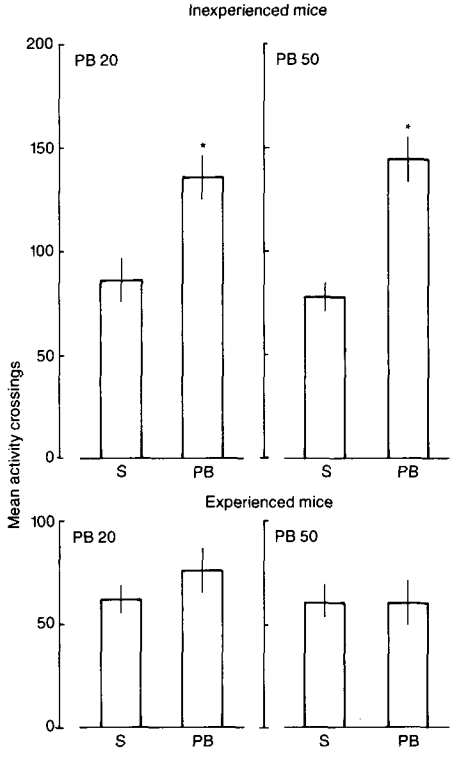


FIG. 1. Locomotor activity measured, during 30 min, in inexperienced or experienced mice, starting 15 min after the administration of a subhypnotic dose of pentobarbitone sodium (20 mg $kg^{-1}$; PB 20) or 15 min after the recovery of the righting reflex, in animals receiving a sleep inducing dose of the barbiturate (50 mg $kg^{-1}$; PB 50). Columns represent mean activity crossings, in groups of 8 mice; vertical lines indicate s.e.m. Asterisk denotes a significant difference ($P < 0{\cdot}05$; Duncan test) between drug treatments and respective controls (saline; S).

It was already well known that the locomotor effects, either stimulatory (Steinberg et al 1961; Rushton et al 1963; Oliverio & Castellano 1974) or depressant (File 1973), exerted by various drugs in animals exposed to a novel environment, can be less expressed or absent in a familiar test apparatus. Thus, the interaction between past experience and the effect of the subhypnotic dose of pentobarbitone was not unexpected. Conversely, the interaction between past experience and posthypnotic hyperactivity was not so predictable, since the past experience could have been distorted by barbiturate anaesthesia.

The stimulant effect of barbiturates on locomotor activity, attributable to their disinhibitory action, may be influenced by various experimental conditions (Watzman et al 1968). It seems now that pentobarbitone-induced locomotor stimulation, as other cases of drug-induced hyperactivity (Steinberg et al 1961), may be evoked by the exteroceptive stimuli provided by a novel environment. The present results demonstrate that susceptibility to such stimuli is again present, in inexperienced mice, soon after recovery from barbiturate anaesthesia. On the other hand, the lack of posthypnotic hyperactivity in experienced animals demonstrates that mice recovering from anaesthesia maintain the knowledge of the environment acquired during past experience.

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