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Enhanced Suppressive Effects of Aversive Events Induced in Rats by Picrotoxin: Possibility of a GABA Control on Behavioral Inhibition

PHILIPPE SOUBRIE, MARIE-HÉLÈNE THIEBOT AND PIERRE SIMON

Unité 19 de Recherches de Neuropsychopharmacologie de l'I N.S.E R M
2, rue d'Alésia, F 75014 Paris, France

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SOUBRIE, P , M H THIEBOT AND P SIMON *Enhanced suppressive effects of aversive events induced in rats by picrotoxin Possibility of a GABA control on behavioral inhibition* PHARMAC BIOCHEM BEHAV **10**(4) 463–469, 1979 —The effects of a GABA receptor blocking agent, picrotoxin (0 5 and 1 mg kg^{-1} IP 30 min before testing) on the behavioral inhibition induced in rats by (1) novelty, (2) punishment or (3) non-reward were studied Picrotoxin was found to enhance (1a) inhibition of water drinking in rats placed for the first time in an unfamiliar box, (1b) inhibition of milk drinking in rats faced with milk for the second time and (1c) inhibition of chocolate intake in rats faced with chocolate for the third time This drug failed to further reduce milk drinking or chocolate intake in rats faced for the first time with milk or chocolate Picrotoxin has no effect on drinking of rats habituated to the test box or familiarized with chocolate Picrotoxin was found to enhance (2) the inhibition of lever presses for food induced in separate groups of rats by the delivery of an electric shock at each 25th or 10th press This drug failed to further reduce depressed pressing induced by the delivery of a shock at each 5th press Picrotoxin did not modify responding in non-punished rats Picrotoxin was found (3a) to impair recovery from reward depression induced in rats by shift from a CRF to a FR 4 schedule but was ineffective on rats well adapted to a FR 4 schedule and (3b) to further depress reduced responding during the first extinction sessions but not during the last extinction session It is proposed that picrotoxin only increases the inhibitory control of aversive events on behavior Since we found this drug to be ineffective on very strong inhibition, one can suggest that GABA may be critically implicated in the release of behavior from mild inhibitory control exerted by novelty, punishment or non-reward

Behavioral inhibition Novelty-induced inhibition Punishment-induced inhibition Non-reward induced inhibition Picrotoxin GABA

NUMEROUS experiments have been devoted to the investigation of a possible involvement of different putative neurotransmitters such as acetylcholine [18, 23, 25], norepinephrine [14, 24, 33], and 5-hydroxytryptamine [26, 31, 34], in the control of the behavioral inhibition induced in animals by punishment or by frustrative non-reward

Recent biochemical and electrophysiological data suggest that benzodiazepines, drugs known to release responding suppressed by various inhibitory influences such as novelty, punishment, and non-reward [9,14], may exert a facilitatory role on neuronal processes involving gamma-aminobutyric acid (GABA) as a transmitter [6, 11, 15, 16, 17] Furthermore, it has been shown that those brain structures known to be involved in behavioral inhibition and considered as major sites of action of minor tranquilizers, namely the amygdaloid complex, septal nuclei and hippocampus, exhibit high L-glutamate decarboxylase activity and/or high levels of GABA [1, 2, 10]

The present work attempts to investigate in rats the possible involvement of the putative inhibitory neurotransmitter, GABA, in the control of behavioral inhibition For that purpose, the effects produced by picrotoxin, a drug known to reduce GABA functioning [6, 15, 36], were investigated on the following forms of behavioral inhibition·

(I) Novelty-induced inhibition· reduced drinking or eating induced by the "neophobic" value (a) of the situation to which the rat had been introduced, (b) of the drink or (c) of the food presented,

(II) Punishment-induced inhibition. decreased lever presses for food produced by pairing some responses with the delivery of electric shocks according to a conflict schedule

(III) Non-reward-induced inhibition: (a) depression of reward in rats shifted from continuous reinforcement to a 4 response fixed-ratio (FR 4) schedule of pellet delivery; (b) reduced lever presses under extinction.

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METHOD

The experiments were carried out on male Wistar A.F rats (230 ± 10 g) housed in groups of 10 under standard conditions (light/dark cycle=12 hr/12 hr, room temperature=21 ± 1°C)

The injections were performed intraperitoneally (picrotoxin being injected as a suspension with acacia gum) in a volume of 0.5 ml/100 g of body weight, 30 min before testing At this time picrotoxin markedly affects GABA processes [15] According to preliminary studies, picrotoxin (purchase from SIGMA) was studied at 2 dosage levels· 0.5 (pH=5 65) and 1 mg kg^{-1} (pH=5 55), 2 mg kg^{-1} was not extensively studied since at this dosage picrotoxin markedly reduced food or water intake or pressing for food Control animals received distilled water injections intraperitoneally Previous experiments using such procedures have shown that acacia gum injections have no effects as compared to water injections

Separate groups of 8 to 12 rats were randomly assigned to an experimental condition All the experiments were carried out in a blind procedure Statistical analysis was done using either a Student's *t*-test or analysis of variance. Normal distribution, in particular for the time spent drinking, was statistically verified by Shapiro's W test [28].

I Novelty-induced Inhibition

(a) Drinking time in non-familiar vs familiar test box The general procedure used has been previously described [29]. The rats were deprived of water (food was freely available) during the 16 hr preceding the test session and were individually placed in a wire-mesh box (36×36×30 cm) A drinking bottle was located in each corner of the box. The orifice of the drinking tube was 3 cm above the floor The time spent drinking by the rats was noted by visual observation and recorded to the nearest second by means of a stop-watch.

All the experiments were performed between 9 and 12 a m

- Water drinking time in a non-familiar box. Three groups of rats injected with picrotoxin 0 5, 1 mg kg^{-1} or distilled water, respectively, were scored for drinking time (5 min and 10 min after their placement in the box) upon their first experience with the experimental box
- Water drinking time in a familiar box Before the test session, rats were subjected to 6 (1 daily trial) 10 min placements in the wire-mesh box. The rats were deprived of water during the 16 hr preceding each trial and, except during the 10 min daily drinking sessions, water was only available between 2 and 6 p m. Three groups of rats, matched according to their drinking time on the 6th trial, were injected with picrotoxin 0 5, 1 mg kg^{-1} or distilled water, respectively, and were tested at their 7th exposure in the box. The time spent drinking 5 and 10 min after the placement of the rat in the box was recorded

(b) Drinking time of non-familiar vs familiar drink Rats were previously habituated (6 trials) to drink water in the wire-mesh box, as described above. After the 6th trial, 6 groups of rats matched according to their drinking time were formed Group 1 was injected with distilled water before each trial and was subjected to two additional water drinking trials (these will be referred to as baseline condition) For the 5 other groups, a non-familiar drink, milk, was substituted for water Group 2 was injected with distilled water before each drinking trial; Groups 3 and 4 were injected with picrotoxin 0.5 and 1 mg kg^{-1}, respectively, before the first experience with milk (7th trial); Groups 5 and 6 were injected with picrotoxin 0.5 and 1 mg kg^{-1}, respectively, before their second experience with milk (8th trial). The time spent drinking (water or milk) within the first 5 min during the 7th and 8th trials was recorded

(c) Food intake of non-familiar vs familiar food The rats were deprived of food (water was freely available) during the 16 hr preceding each of the 6 trials Each trial (1 daily trial between 9 and 12 a.m.) was run for 30 min in a 42×28×18 cm translucent box on the floor of which 6 chow biscuits were placed. Except for the 30 min daily sessions, food was only available between 2 and 6 p m. After the 6th trial, 7 groups of rats matched according to their food intake were formed Group 1 was injected with distilled water before each trial and subjected to 3 additional standard food intake sessions (baseline). For the other 6 groups, a non-familiar food, chocolate, was substituted for standard food Group 2 was injected with distilled water before each trial. Groups 3 and 4 were injected with picrotoxin 0.5 and 1 mg kg^{-1}, respectively, before their 1st experience with chocolate (7th trial), Groups 5 and 6 were injected with picrotoxin 0.5 and 1 mg kg^{-1}, respectively, before their 3rd experience with chocolate (9th trial); Group 7 was injected with picrotoxin 1 mg kg^{-1} before its 6th experience with chocolate (12th trial)

The amount of food ingested was evaluated by weighing the chow biscuits or the pieces of chocolate before and after the 30 min food intake session The amount spilled, which was very low over a 30 min period, was collected and weighed with the remaining pieces of food and was not modified by the drugs studied

Experiments conducted on punishment- and non-reward-induced inhibition were performed using 3 operant chambers (housing in ventilated sound attenuated cubicles) with an automatic magazine delivering 45 mg Noyes pellets The boxes were equipped with levers (5.5 cm above the grid floor) which required a vertical force of at least 12 g to operate the microswitch. Rewards were delivered into a recess between the 2 levers in front of which was a 5 cm wide transparent flap which the rat had to push open with its head to collect pellets. General illumination came from a 2 8 W houselight on the ceiling. Electric current could be passed through the grid floor which consisted of 0 5 cm dia stainless steel rods spaced 0 5 cm apart

The rats, maintained at 80–85% of their normal body weight, were given 10 trials (1 daily session of 15 min) to press the right lever of the Skinner-box to obtain food pellets according to a continuous reinforcement schedule Rats not displaying a high level of responding (80 presses/15 min) at the end of these sessions were discarded. In order to establish the baseline level of lever presses, 6 additional daily sessions were performed Each rat was tested at the same time of the day After the 6th session, separate groups of rats, matched according to their performance, were formed and subjected to one of the experimental sessions

II Punishment-induced Inhibition

For 8 groups of rats, the experimental session modified from the punishment procedure used by Glowa and Barrett [12] consists of reinforcing each press with the delivery of a pellet and in pairing some presses with the delivery of an electric shock (0 35 mA) through the grid floor of the Skinner box. Groups 1 and 2, injected with distilled water and picrotoxin 1 mg kg^{-1}, respectively, were punished according

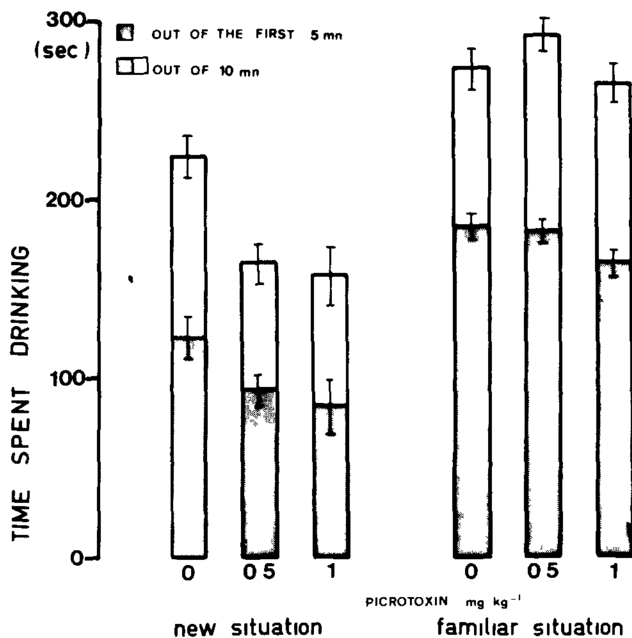

FIG 1 Effects of picrotoxin on novelty (unknown box)-induced inhibition of water drinking in rats The columns refer to the time (sec) spent drinking (mean ± SEM) by rats 5 and 10 min after their placement in the box Novelty-induced inhibition can be evaluated by comparing the drinking time of control rats tested for the first time in the box to that of rats tested after six 10 min daily drinking sessions in the box

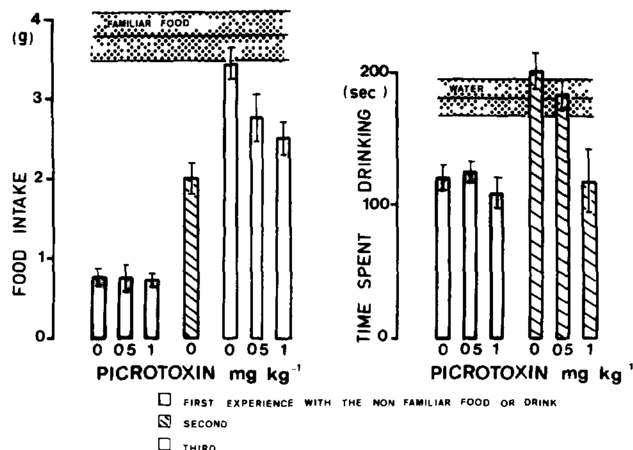

FIG 2 Effects of picrotoxin on the consumption of a "neophobic" food (chocolate) or drink (milk) in rats The columns refer left part to 30 min food intake (g) (mean ± SEM) or right part to 5 min drinking time (sec) (mean ± SEM) Novelty-induced inhibition can be evaluated by comparing the levels of performance of control rats when tested either with their familiar food or drink (baseline), at their first experience or after previous experience(s) with chocolate or milk For each trial, separate groups of picrotoxin treated rats were used

to a 5 response fixed-ratio schedule (FR 5) of shock presentation (each 5th press produced a shock), Groups 3, 4 and 5, injected with distilled water, picrotoxin 0.5 and 1 mg kg^{-1}, respectively, were punished according to a FR 10 schedule of shock presentation, Groups 6, 7 and 8 injected with distilled water, picrotoxin 0.5 and 1 mg kg^{-1}, respectively, were punished according to a FR 25 schedule of shock presentation.

Two groups (Groups 9 and 10, injected with distilled water and picrotoxin 1 mg kg^{-1}, respectively) were not subjected to a conflict schedule and the experimental session was identical to the 6 previous sessions.

III Non-reward-induced Inhibition

(a) Shift from continuous reinforcement to a FR 4 schedule of reward delivery Rats were trained to perform in the Skinner box according to a continuous reinforcement schedule, as described above After the 6th additional session, 11 groups of rats were subjected to daily FR 4 experimental sessions Group 1 was given distilled water before each FR 4 session. Groups 2 and 3 were injected with picrotoxin 0.5 and 1 mg kg^{-1}, respectively, before the 1st FR 4 session, Groups 4 and 5 were injected with picrotoxin 0 5 and 1 mg kg^{-1}, respectively, before the 2nd FR 4 session, Groups 6 and 7 were injected with picrotoxin 0.5 and 1 mg kg^{-1}, respectively, before the 3rd FR 4 session; Groups 8 and 9 were injected with picrotoxin 0 5 and 1 mg kg^{-1}, respectively, before the 4th FR 4 session; Groups 10 and 11 were injected with picrotoxin 0.5 and 1 mg kg^{-1}, respectively, before the 7th FR 4 session.

(b) Extinction of food-reinforced responding Rats were trained to perform in the Skinner box according to a continuous reinforcement schedule, as described above After the 6th additional session, 7 groups of rats were subjected to daily experimental extinction sessions Group 1 was injected with distilled water before each session Groups 2 and 3 were injected with picrotoxin 0 5 and 1 mg kg^{-1}, respectively, before the 1st extinction session; Groups 4 and 5 were injected with picrotoxin 0 5 and 1 mg kg^{-1}, respectively, before the 2nd extinction session; Groups 6 and 7 were injected with picrotoxin 0 5 and 1 mg kg^{-1}, respectively, before the 3rd extinction session

RESULTS

I Novelty-induced Inhibition

(a) Water drinking in familiar vs non-familiar box As compared to rats placed in a familiar situation, control rats placed in a non-familiar situation (first experience with a wire-mesh box) exhibited a statistically ($p<0.01$) significant lessened drinking time either over 5 ($t=4.299$) or 10 min ($t=2.946$) (Fig. 1). Picrotoxin reduced in a dose-related manner the drinking time (over 10 min) of rats placed in a non-familiar situation (linear regression· $F(1,27)=9.024$, $p<0$ 01) but did not exhibit such an effect on the drinking time over the first 5 min (linear regression $F(1,27)=3$ 806, NS). Picrotoxin had no significant effect on drinking time of rats placed in a familiar situation (Fig. 1)

(b) Drinking time of familiar vs. non-familiar drink As compared to rats faced with a familiar drink (water), or to rats faced with milk for the second time, control rats faced with milk for the first time showed a statistically ($p<0$ 01) significant lessened drinking time ($t=3$ 605 and $t=4$ 807, respectively) (Fig. 2). Picrotoxin had no significant effect in rats faced with milk for the first time but reduced in a dose-related manner (linear regression $F(1,24)=9$ 908, $p<0$ 01) the milk drinking time of rats at their second trial

(c) Food intake of familiar vs non-familiar food As compared to rats faced with a familiar food, control rats faced

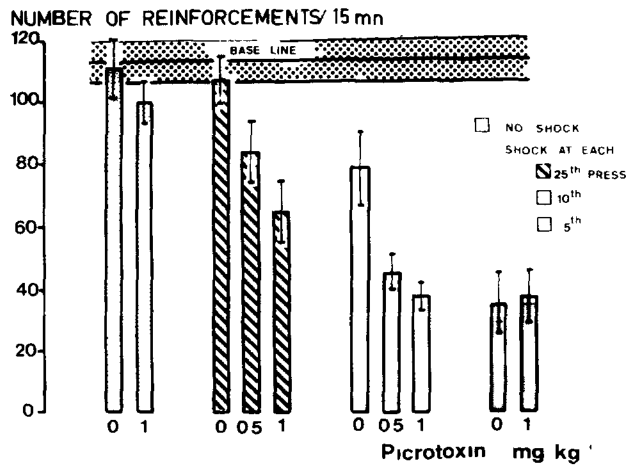

FIG 3 Effects of picrotoxin on responding of rats under 3 different conflict conditions The columns refer to the number of pellets (mean ± SEM) obtained by different groups of rats over a 15 min non-conflictual or conflictual session Responding was rewarded according to a continuously reinforced schedule and (conflictual sessions) punished at each 25th, 10th or 5th presses by an electric shock (0 35 mA) Baseline refers to the number of pellets (mean ± SEM) obtained by the rats during the last training sessions

with chocolate for the first time exhibited a highly reduced food intake (Fig 2) The amount of chocolate ingested increased over each successive trial to reach 5.46 ± 0 67 g at the 6th trial with chocolate Picrotoxin had no significant effect on chocolate intake during the 1st trial with chocolate and decreased in a dose related manner (linear regression. $F(1,21)=6\ 332$, $p<0.02$) chocolate intake during the 3rd trial with chocolate (Fig 2) Picrotoxin 1 mg kg^{-1} administered before the 6th trial had no significant effect on chocolate intake (5 09 ± 0 59 vs 5 46 ± 0 67 g)

A control experiment was carried out in order to study the effects of two other convulsants (bemegride and strychnine given IP 30 min before testing) on drinking time in a familiar vs non-familiar box Bemegride significantly ($p<0\ 02$) reduced the drinking time (over 10 min) of rats placed in a non-familiar box (4 mg kg^{-1} 77%, 8 mg kg^{-1} 71% of control values). This drug significantly reduced ($p<0.02$) the drinking time of rats placed in a familiar situation (4 mg kg^{-1} 77%, 8 mg kg^{-1} 77% of control values) Strychnine at 1 5 mg kg^{-1} significantly reduced the drinking time of rats placed in a non-familiar box (77% of control values, $p<0\ 01$) and the drinking time of rats placed in a familiar situation (89% of control values, $p<0\ 05$)

In a pilot experiment, picrotoxin was found to raise in a dose-related manner the aversive value of water adulterated with picric acid (3%) (time spent drinking over 5 min controls=115 ± 7 sec, picrotoxin 0 5 mg kg^{-1}=94 ± 9 sec, picrotoxin 1 mg kg^{-1}=88 ± 7 sec, linear regression $F(1,24)=6\ 323$, $p<0\ 02$).

II Punishment-induced Inhibition

In controls, the number of lever presses made during 15 min was found to be directly related to the ratio of punished responses (Fig. 3) Responding was not statistically reduced when the electric shocks were delivered at each 25th press, statistically reduced ($t=2\ 447$, $p<0\ 05$) when the electric shocks were delivered at each 10th press and almost suppressed ($t=6\ 390$, $p<0.01$) when the shocks were delivered at each 5th press Picrotoxin 0 5 and 1 mg kg^{-1} was found to reduce in a dose-related manner responding of rats subjected to the FR 25 or to the FR 10 punished schedule (linear regression $F(1,27)=8.260$, $p<0\ 01$, $F(1,27)=6\ 476$, $p<0\ 02$ respectively) Pircotoxin 1 mg kg^{-1} did not statistically modify the depressed responding of rats subjected to the FR 5 punished schedule Picrotoxin 1 mg kg^{-1} failed to significantly reduce ($t=1\ 313$) the number of pellets obtained by non-punished rats (Fig 3)

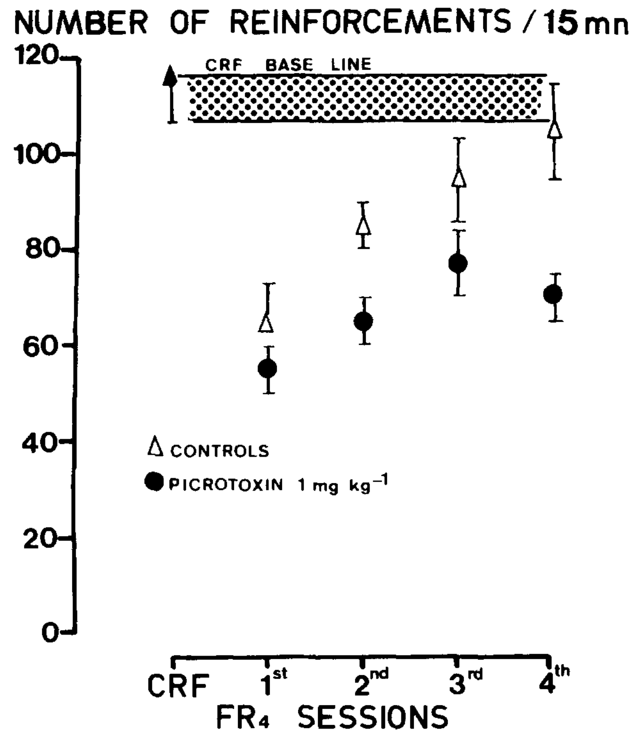

FIG 4 Effects of picrotoxin on the depression of rewards induced by shift from CRF to FR 4 schedule and on recovery of baseline under successive daily FR 4 sessions Results refer to the number of pellets (mean ± SEM) obtained by different groups of rats over a 15 min FR 4 session Baseline refers to the number of pellets (mean ± SEM) obtained during the last CRF training sessions For each FR 4 session, separate groups of picrotoxin treated rats were used

In a pilot study, picrotoxin 1 mg kg^{-1} was found to raise the suppressive effect of a 3 min presentation of a light stimulus previously associated with non-contingent electric shocks (number of lever presses during these 3 min controls=15 2 ± 1 5, picrotoxin 1 mg kg^{-1}=11 7 ± 0 7, $t=2\ 203$, $p<0\ 05$)

III Non-reward-induced Inhibition

(a) Shift from continuous reinforcement to FR 4 schedule

During the first FR 4 session, despite an increased rate of lever pressing, control rats obtained a statistically ($t=4\ 235$, $p<0\ 01$) significant lessened number of reinforcements as compared to that obtained during the previous continuously reinforced sessions (Fig. 4) The number of pellets obtained increased over successive FR 4 sessions to reach 138 ± 8 at the 7th FR 4 session ($t=1\ 827$, not statistically different from

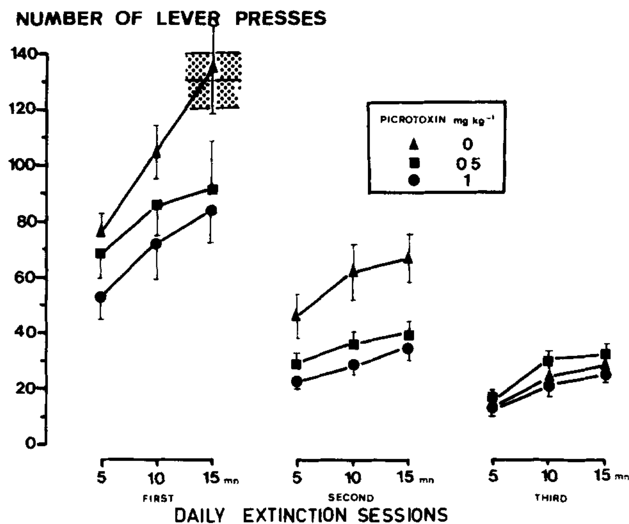

FIG 5. Effects of picrotoxin on responding of rats under 3 successive daily extinction sessions Results refer to a 5 min cumulated number of lever presses (mean ± SEM) during a 15 min session Baseline (dotted area) refers to the 15 min number of lever presses (mean ± SEM) performed during the last continuously rewarded sessions For each extinction session, separate groups of picrotoxin-treated rats were used

baseline). Picrotoxin, over the 4 FR 4 sessions, induced a dose-related decrease of the number of presses emitted during 15 min periods (linear regression: $F(1,127)=4\ 821$, $p<0.05$) although this effect was not equally marked for each session (Fig. 4). Picrotoxin given before the 7th FR 4 session did not significantly reduce responding during this session (138 ± 8 vs. 132 ± 6 reinforcements)

(b) Extinction of food reinforced responses. In control rats, failure to reinforce responses induced no decrease in responding during the 1st 15 min extinction session but induced markedly reduced responding as compared to baseline ($t=3.841$, $p<0.01$) during the 2nd 15 min extinction session and an almost suppressed responding during the 3rd 15 min extinction session (Fig 5). Picrotoxin 0.5 or 1 mg kg^{-1}, when given before either the 1st or the 2nd extinction session reduced responding during these sessions in a statistically significant dose-related manner (linear regression for the number of presses during 15 min first session $F(1,27)=6\ 178$, $p<0\ 02$; second session $F(1,33)=7.552$, $p<0.01$) This drug given before the 3rd extinction session failed to significantly modify responding during this session (Fig 5).

DISCUSSION

In the present experiment, various forms of behavioral inhibition were elicited in rats, either by novelty, punishment or frustrative non-reward. These three major factors are known to exert a suppressive effect on positively reinforced responding [8, 9, 14, 29]. Such a suppressive effect was defined by comparing the level of responding of rats subjected to aversive situations to that of rats either slightly or not subjected to the inhibitory events.

As expected, the magnitude of the behavioral suppression could be directly related to the strength of the inhibitory factors "neophobic" responses decreased over time as familiarization was increased; punishment-induced suppression was enhanced as shock density was augmented, non-reward induced suppression increased over time as reward expectance was decreased.

The effects of picrotoxin on forms of behavioral inhibition differing in their inducing factors and (for a given factor) in their intensity were therefore investigated

Picrotoxin at infra-convulsant doses was found to magnify the response suppression induced by either novelty, punishment, non-reward or bitter taste. Inhibition of water drinking induced by the introduction of the animals into unfamiliar surroundings was more marked during the first 5 min than during the last 5 min of the test This fact may be explained both by an attenuation of the "neophobia" of naive animals over the 10 min of the test, and by the development of satiety mechanisms which limit drinking in habituated animals Thus, the interaction of these two factors did not allow us to clearly specify the magnitude of the effects of picrotoxin on the inhibition of water drinking Nevertheless, the foregoing results suggest that picrotoxin enhances the suppressive value of aversive events.

For this proposal to be valid, it was necessary to demonstrate that picrotoxin exerts a specific effect on behavioral suppression, i e , enhances inhibition induced by novelty, punishment or non-reward, without affecting behavior under non-aversive conditions Picrotoxin (i) reduced punished responding but had no effect on non-punished responding in a conflict situation, (ii) depressed food or water intake in naive rats but failed to exhibit such an effect in experienced animals, (iii) reduced lever pressing for food during the first sessions following the shift from CRF to FR 4 but left unchanged responding in animals well adapted to the FR 4 schedule In agreement with previous reports [4,31], these data suggest that picrotoxin at the doses used acted preferentially on behavior suppressed by aversive events, and only when doses were increased (2 mg kg^{-1}) did picrotoxin also alter responding under non-aversive conditions. These same data argue against the hypothesis that the intensifying effect of picrotoxin on the inhibition of food- (or drink-) related responses studied resulted from a decreased motivation The effect of picrotoxin in our conflict procedure may result from enhanced pain induced by electric shock. This may be related to the GABA-antagonistic activity of picrotoxin [6, 15, 36] since GABA processes have been implicated in pain control [3,7].

However, the preferential action of picrotoxin on behavior under aversive conditions could result from a greater drug sensitivity to the responses studied under aversive conditions as compared to those studied under control conditions.

Accordingly, under our experimental conditions, one can suggest that picrotoxin affected suppressed behavior only in that suppressed behavior consists of not fully acquired responses which are known to exhibit a greater drug sensitivity as compared to already established responses However, no data strongly support the fact that picrotoxin exhibits a consistent effect inversely correlated with the level of training of the animals picrotoxin (i) decreased water intake in naive rats but not chocolate or milk intake although it depressed chocolate or milk intake during the subsequent sessions, (ii) failed to markedly depress responding during the first FR 4 session while a pronounced depressant effect was found during the 4th FR 4 session and (iii) may improve timing behavior in rats well adapted to a DRL schedule [30]. Conversely, the effects of picrotoxin on extinction and on taste aversion

could suggest that this drug may improve some learning abilities, as found after pretrial administration of various convulsants [22]. Finally, it is generally assumed that FR responding is more sensitive to drugs than CRF responding Such a differential sensitivity could not explain why the depressant effect of picrotoxin on FR 4 responding was restricted to the FR 4 sessions that follow the shift from CRF to FR 4 schedule

The data presented here suggest that at low dosage levels picrotoxin could specifically affect behavior controlled by aversive events Although it has been found that picrotoxin interacts with other neuronal systems [11, 15, 19, 20, 27, 35], this drug has been shown to reduce GABA functioning [6,15] probably by impairing GABA-associated chloride conductance changes [36] Unlike picrotoxin, bemegride and, as already presented [31], although contradictory results have been reported [21], strychnine did not seem to preferentially affect behavior under aversive conditions All these data indicate that a pre-convulsive state *per se* is not sufficient to enhance the inhibitory effects of aversive events and may be suggestive of a GABAergic control of behavioral inhibition Accordingly, brain structures which have been implicated in the processes associated with response suppression, especially the amygdaloid complex, septal nuclei and hippocampus, showed high GABA levels and/or exhibited high L-glutamate decarboxylase activity [1, 2, 10] Furthermore, benzodiazepines, drugs assumed to exert a facilitatory role on GABAergic processes [6, 11, 15, 16, 17], are known to release behavior from inhibitory influences [9]. Although contradictory reports [21] have been presented, such an effect was found in some experimental situations antagonized by picrotoxin [4,31] Taken together, these data provide strong support for the possibility of a GABAergic control of behavioral inhibition

As indicated by our results, picrotoxin generally failed to enhance strong inhibition This suggests, in agreement with recent biochemical data [13], that GABA seems critical only in maintaining responding under mildly aversive conditions and/or in the adaptation of the animals to aversive events

After peripheral administration, picrotoxin (at doses very nears to ours), has been shown to alter various parameters related to the activity of GABAergic systems [4,15] However, from our data, it is not possible to ascertain whether or not the intensifying effects of picrotoxin on behavioral inhibition derive only from its blockade of GABA transmission However, GABA mimetics such as aminooxyacetic acid [5], a blocker of GABA transaminase, or muscimol [32] a direct GABA agonist, failed in most aversive situations to exert a benzodiazepine-like activity or to exert opposite effects to those obtained with picrotoxin. This may suggest, as stated by Haefely [16] that "while benzodiazepines require the activity of GABAergic neurones in order to produce their effects, GABA mimetics will stimulate GABA receptors irrespective of the activity of GABA neurones and even receptors on target cells that are normally not activated by endogenous GABA". These same data may indicate a critical interaction of picrotoxin with non-GABAergic systems Indeed, picrotoxin has been reported to interfere with various neuronal systems [11, 15, 19, 20, 27, 35], many of which are implicated in the control of behavioral inhibition cholinergic systems [18, 23, 25], 5-hydroxytryptamine systems [26, 31, 34] and noradrenergic systems [14, 24, 33] Nevertheless, except in a few reports [27,35], the activity of picrotoxin upon such neuronal systems had been reported to be secondary to its effects on GABA processes linked with these neuronal systems [11, 15, 19, 20] Since the foregoing reports preferentially implicate 5-hydroxytryptamine neurons in punishment-induced inhibition and norepinephrine neurons in non-reward-inhibition (processes involved in responses to novelty have not been yet clearly identified), one can suggest that the activity of these neurons, as indicated by our results, may be indirectly regulated or inhibited by GABA-containing neurons In particular, during punishment, the relationship between GABA and 5-hydroxytryptamine-containing raphe cells that are probably involved in response suppression [34] can be tentatively discussed by considering recent electrophysiological data demonstrating a GABA control of these raphe cells [11] Picrotoxin alone was unable to alter the activity of the raphe cells while, when GABA control was activated, picrotoxin markedly affected their activity. Accordingly, the selective effects of picrotoxin we observed on punished responding as compared to nonpunished responding, may be explained by speculating that GABA control of 5-hydroxytryptamine cells, not present under non-punished conditions, could be triggered by punishment

Although further experiments are required before concluding that picrotoxin selectively affects response suppression through a GABAergic mechanism, our data suggest that GABAergic processes may be involved in the release of behavior from mild inhibitory influences.

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