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Psilocybin: Biphasic Dose-Response Effects on the Acoustic Startle Reflex in the Rat[1]

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DAVIS, M. AND J. K. WALTERS. *Psilocybin: Biphasic dose-response effects on the acoustic startle reflex in the rat.* PHARMAC. BIOCHEM. BEHAV. 6(4) 427–431, 1977. – The startle reflex was measured in 7 groups of 10 rats each after intraperitoneal injection of saline or 0.25, 0.50, 0.75, 1.0, 2.0, 4.0 or 8.0 mg/kg psilocybin. Low doses (0.75–2.0 mg/kg) increased startle amplitude whereas high doses (4.0–8.0 mg/kg) depressed startle. Selected low (0.71 mg/kg) or high (5.70 mg/kg) doses of psilocin also had a biphasic dose-response effect on startle comparable in magnitude to equimolar doses of psilocybin. This biphasic dose-response relationship of the indole hallucinogen, psilocybin, on startle is consistent with the hypothesis that startle is increased when the firing rates of midbrain raphe neurons are selectively inhibited but is depressed when neurons postsynaptic to raphe cells are also inhibited.

Startle Psilocybin Psilocin Hallucinogens

CONSIDERABLE evidence suggests that the acoustic startle reflex in the rat is normally inhibited by the raphe-serotonin system. Electrolytic lesions of the midbrain raphe nuclei increase startle amplitude [11,18]. Inhibition of serotonin (5-HT) synthesis by p-chlorophenylalanine [7] or p-chloroamphetamine [14] also increases startle amplitude. Conversely, increased release of 5-HT shortly after administration of p-chloroamphetamine [14] or direct infusion of 5-HT intraventricularly [19] or into specific areas of the brain [17], depress startle.

It is also known that low doses of drugs which are hallucinogens in man alter startle in the rat. For example low doses of d-lysergic acid diethylamide (LSD) or N-N-dimethyltryptamine (DMT) increase startle [12, 13, 26]. Increased startle after low doses of LSD or DMT has been attributed to the fact that low doses of these compounds directly and specifically inhibit the firing rate of midbrain raphe neurons [2, 3, 20], and thereby reduce the release of 5-HT [16]. Functionally, low doses of indole hallucinogens act like serotonin antagonists.

It is also known, however, that higher doses of hallucinogens directly inhibit neurons postsynaptic to raphe cells [3,20]. Functionally, high doses of indole hallucinogens act like serotonin agonists.

Behaviorally therefore, low doses of hallucinogens should have effects exactly opposite to those of high doses, provided that the behavior under study is modulated by the raphe-serotonin system. Consistent with this is the finding that low doses of DMT increase startle amplitude whereas high doses decrease startle amplitude [13]. In addition, biphasic dose-response effects of hallucinogens have been observed in a variety of other behavioral situations which may also be modulated by 5-HT [4, 5, 8, 23, 24, 25, 27, 28, 29].

The purpose of the present study was to test the generality of this relationship using another hallucinogen, psilocybin. Low doses of the drug psilocin, which most probably is the active metabolite of psilocybin [1, 21, 22, 31] have been shown to inhibit the firing rate of the presynaptic raphe neurons, whereas higher doses are required to inhibit neurons postsynaptic to the raphe [3]. Based on these differential pre- and postsynaptic 5-HT effects, psilocybin should also have a biphasic dose-response effect on startle.

EXPERIMENT 1: EFFECTS OF VARIOUS DOSES OF PSILOCYBIN

Method

Animals. A total of 70 male albino Sprague Dawley rats weighing between 250–300 g were used. Upon receipt from the supplier (Charles River Co.) the rats were housed in group cages of 4–5 rats each in a large colony room that was maintained on a 12–12 light-dark schedule. Food and water were continuously available.

Apparatus. The apparatus has been described in detail elsewhere [30]. Briefly, 5 separate stabilimeters were used to record the amplitude of the startle response. Each stabilimeter consisted of an 8 × 15 × 15 cm Plexiglas and wire mesh cage suspended between compression springs within a steel frame. Cage movement resulted in displacement of an accelerometer where the resultant voltage was

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proportional to the velocity of displacement. Startle amplitude was defined as the maximum accelerometer voltage that occurred during the first 200 msec after the startle stimulus was delivered and was measured with a specially designed sample and hold circuit. The stabilimeters were housed in a dark, ventilated, sound attenuated chamber, 1.1 m from a high-frequency speaker. The startle stimulus was a 4000 Hz, 90 msec, 120 db tone having a rise-decay time of 5 msec. Background white noise, provided by a white noise generator, was 46 db. Sound level measurements were made within the cages with a General Radio Model 1551-C sound level meter (A-scale).

Procedure. Prior to drug testing, each of 70 rats was placed in a test cage and 5 min later presented with 10 tones at a 20 sec intertone interval (ITI). Based on the mean startle amplitude across these 10 tones, the rats were divided into 7 groups of 10 rats each, with each group having similar mean levels of startle.

On the first test (2–3 days after the pretest), half the rats in each group were injected intraperitoneally (IP) with isotonic saline (1 ml) and half with psilocybin, using doses of 0.25, 0.50, 0.75, 1.0, 2.0, 4.0 or 8.0 mg/kg. A different matched group was used for each dose. Immediately after the injection the rats were placed in the test chamber and 5 min later presented with 45 tones at a 20 sec ITI. For the higher concentrations, psilocybin was dissolved in saline by adding a few drops of 1 M tartaric acid and then adjusting the pH to 6.0 with 0.1 N NaOH. The lower concentrations were then made by diluting appropriately with saline from this stock solution.

Two days later the same procedure was repeated. This time however, rats previously injected with saline were now injected with psilocybin and vice versa. Thus each rat served as its own control with respect to saline vs. psilocybin, whereas dosage was varied between animals.

Results

Figure 1 shows the percent change in startle after various doses of psilocybin. Percent change was computed as: [(Mean startle amplitude across all 45 tones after psilocybin-mean amplitude after saline)/(Mean amplitude after saline)] × 100. Similar to other hallucinogens, psilocybin had a biphasic dose-response effect on startle. Low doses increased startle, whereas high doses depressed startle. This conclusion was confirmed by an overall analysis of variance on the raw scores which found a significant quadratic dose response relationship, $F(1,63) = 19.17$, $p < 0.001$.

EXPERIMENT 2: EFFECTS OF LOW AND HIGH DOSES OF PSILOCIN

Considerable evidence suggests that psilocybin is rapidly converted to psilocin upon administration, and that psilocin is the active metabolite of psilocybin [1, 21, 22, 31]. If this is so, then doses of psilocin equimolar to those of psilocybin should have comparable effects on startle.

Method

A total of 40 rats were matched into four groups of 10 rats each as previously described. Two days later half the rats in each group were injected with saline and half with either 0.71 or 5.6 mg/kg psilocin. These doses correspond to doses of 1.0 and 8.0 mg/kg psilocybin given the 1:1.4 ratio between the molecular weights of psilocin and

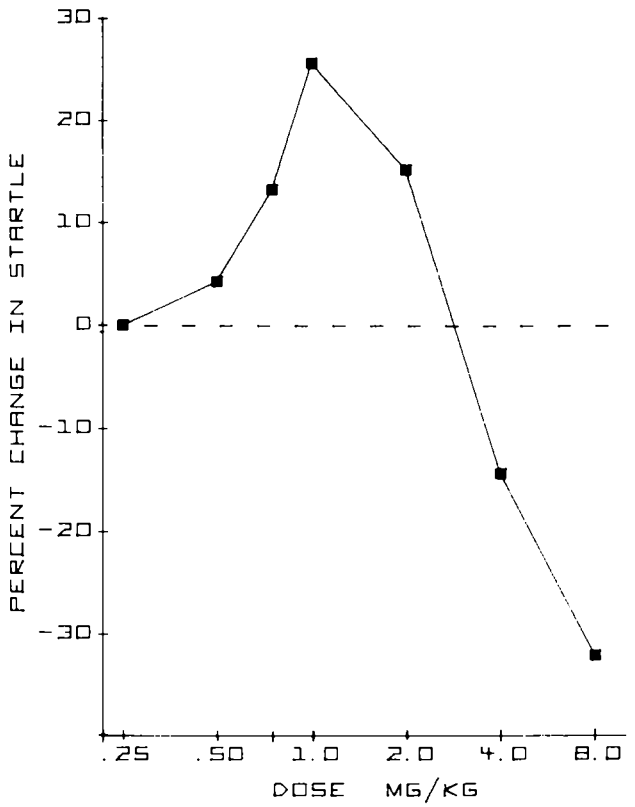

FIG. 1. Mean percent change in startle after injection of various doses of psilocybin relative to an injection of saline.

psilocybin, respectively. Immediately after the injections the rats were placed in the test cages and 5 min later presented with 45 tones at a 20 sec ITI. Two days later the same procedures were repeated, except animals previously injected with saline were now given psilocin and vice versa. Psilocin was dissolved in saline by adding a few drops of 1 N HCl and then adjusting the pH to 6.0 with 0.1N NaOH. Solutions were made up immediately before testing.

Results

At these doses, psilocin also had a biphasic dose-response effect on startle. The 0.71 mg/kg dose increased startle by 24%, $t(19) = 3.68$, $p < 0.005$ whereas the 5.7 mg/kg dose depressed startle by 48% $t(19) = 5.96$, $p < 0.001$. The magnitude of the effects compare reasonably well with the magnitude of equimolar doses of psilocybin in Experiment 1 (25% and −32%).

EXPERIMENT 3: TIME COURSE OF PSILOCYBIN'S ACTION

In Experiment 1, testing after psilocybin was only conducted for a relatively brief period. The purpose of this experiment was to evaluate more exactly the time course of psilocybin's action on startle.

Method

A total of 40 rats were matched into two groups of 20 rats each as previously described. Two days later, half the rats were injected with saline and half with psilocybin (1 mg/kg). Immediately after the injection the rats were

presented with 180 tones at a 20 sec ITI. Two days later the same procedures were repeated except animals previously injected with saline were now given psilocybin and vice versa.

Results

Figure 2 shows the mean amplitude startle response over successive 2 min periods (i.e. over blocks of 6 tones since there were 3 tones/min) after injection of either saline or psilocybin. Consistent with Experiment 1, this dose of psilocybin increased startle over the first 5–20 min of the session and this excitatory effect was statistically significant, $t(19) = 2.64$, $p<0.02$. The magnitude of the excitatory effect over this time period was small but comparable in magnitude to the excitatory effect over a similar time period in Experiment 1 (22% vs 25%).

Startle amplitude in rats injected with saline showed an initial decrease in amplitude followed by an increase over the rest of the session. This pattern is typical when startle-eliciting tones are initiated immediately after placing the rats in the test cages [9]. The general increase in startle across the session reflects the combined influence of habituation to the tones, which serves to decrease startle, and constant exposure to background white noise, which serves to increase startle [9].

Interestingly, psilocybin appeared to attenuate the normal increase in startle toward the end of the session, since the average startle amplitude from 30–50 min was lower after psilocybin than saline, $t(19) = 3.53$, $p<0.001$. Relative to the saline condition, therefore, psilocybin first increased then later depressed startle, leading to a significant Drug × Test Time interaction $F(29,551) = 2.50$, $p<0.001$. Subsequent work with other doses (0.50 and 4.0 mg/kg) indicated that these later depressive effects were dose-related, with higher doses producing greater subsequent depression of startle beginning earlier in the session.

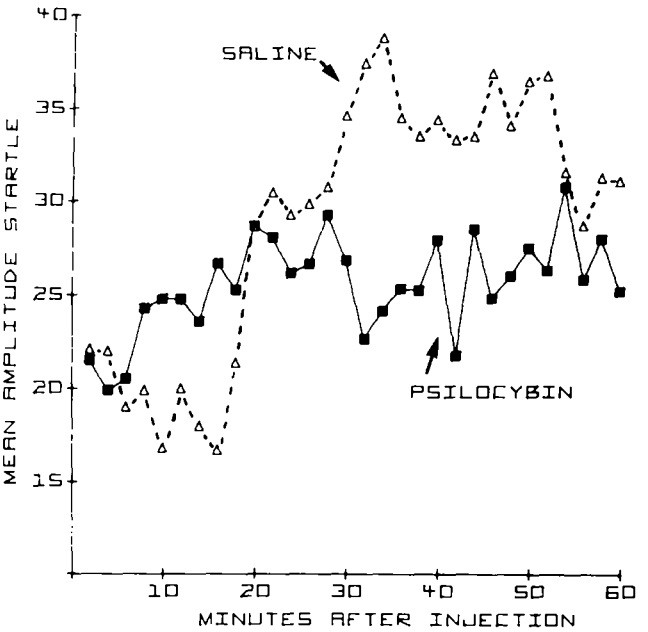

FIG. 2. Mean amplitude startle response over successive 2 min periods after injection of psilocybin (1.0 mg/kg) or saline.

GENERAL DISCUSSION

The present results are consistent with the idea that low and high doses of hallucinogenic drugs should have opposite effects on behaviors which are believed to be modulated by the raphe-serotonin system. Low doses of psilocybin, by inhibiting the firing rate of the presynaptic raphe neurons, should release postsynaptic neurons from inhibitory 5-HT input and increase startle, since startle is believed to normally be inhibited by 5-HT. High doses, by directly inhibiting neurons postsynaptic to the raphe, should act like 5-HT agonists and depress startle.

It could of course be argued that high doses of any drug may depress startle nonspecifically and that this could account for the depressive effect of the higher doses of psilocybin. This possibility cannot be ruled out. However, it is important to note that very high doses of d- or l-amphetamine (16–32 mg/kg) have potent excitatory effects on startle, while other drugs such as clonidine have monotonic depressive effects [10,15]. No hint of a biphasic dose-response relationship is observed with either drug over a wide range of doses.

The fact that equimolar doses of psilocybin and psilocin had similar biphasic dose-response effects on startle supports the idea that the behavioral effects of psilocybin probably result from its conversion to psilocin *in vivo*. Under the test conditions used, no difference was detected in the time course of changes in startle amplitude produced by psilocin or psilocybin. This is consistent with the finding that the time course of each is also very similar in altering human behavior [31]. If psilocybin must be converted to psilocin to be effective, then both human data and now startle data suggest that this conversion occurs very rapidly after administration.

It should be emphasized that the excitatory effect of psilocybin (or psilocin) on startle was small. Even though reproducible in all three experiments and statistically significant, the excitatory effect never exceeded about 30%, regardless of the dose used. Thus psilocybin's effect is similar in magnitude to that of DMT [13], and both drugs are considerably less efficacious than LSD in increasing acoustic startle [12]. In terms of potency, psilocybin was about 2–4 times less potent than DMT in increasing startle but also somewhat less potent in depressing the response [12]. This is inconsistent with human data since psilocybin is generally regarded to be a more potent hallucinogen than DMT in man. Compared to LSD, psilocybin was about 50 times less potent at increasing startle. This is shown by the fact that a dose of 20 μg/kg LSD increases startle amplitude by about 25% from 10–20 min after injection [12], whereas a dose of 1.0 mg/kg psilocybin produced an effect of similar magnitude in the present study. Therefore, the potency ratio of LSD to psilocybin in increasing startle is the same order of magnitude as the 65:1 potency ratio of LSD to psilocybin in altering various psychological measures in man [31].

An unexpected finding was that even at relatively low doses, psilocybin suppressed startle about 30–50 min after injection. If low doses of psilocybin act only by inhibiting the raphe neurons, then one would not expect to see depressive effects on startle at longer times after injection. Instead, one would simply expect to see a dimmunition, or perhaps even an increase in the early excitation as the drug was metabolized and the dose effectively moved to the left on the biphasic dose-response curve. The reasons psilocybin

depresses startle at these later times is not clear. It is interesting in this regard that psilocybin has also been reported to cause an initial increase in general motor activity followed by a period of marked motor inhibition in both rats and mice ([6] reported in [1]).

It has been suggested that the raphe-serotonin system does not inhibit the startle circuit directly, but instead inhibits systems which normally mediate the increase or sensitization in startle produced by conditions such as continuous exposure to background white noice [9, 12, 14]. For example, the usual excitatory effect of low doses of LSD on acoustic startle can be attenuated by reducing the level of background noise in testing [12]. Conversely, the depressive effect of parachloroamphetamine (PCA) seen shortly after administration, at a time when PCA releases serotonin, can also be attenuated by reducing the level of background noise [14]. Rather than simply affecting the motor expression of startle, drugs thought to alter 5-HT function appear to alter the relative saliency of surrounding environmental conditions, which in turn alter startle. It is interesting in this regard that the depressive effect of psilocybin toward the end of the session may have resulted from a decrease in the sensitization which normally is seen under these test conditions.

It is conceivable, therefore, that even low doses of psilocybin depress the firing rates of neurons postsynaptic to the raphe, which should serve to inhibit sensitization. However, this particular drug-receptor interaction may take a considerable time to occur. A peculiarity of the psilocybin metabolite, psilocin, is that it does take an unusually long time to depress both raphe and postsynaptic raphe neuronal firing rates relative to other indole hallucinogens (G. K. Aghajanian, personal communication). Another possibility is that psilocybin, after being converted to psilocin, is demethylated to 4-hydroxytryptamine in the brain (G. K. Aghajanian, personnel communication). This compound does have a potent and unusually long lasting depressive effect on neurons postsynaptic to the raphe when given via iontophoresis [3], and could account for the long lasting depressive effect of psilocybin on startle.

In summary, the present results provide further evidence that the behavioral effects of hallucinogenic drugs are critically dependent on the exact dose that is used. Since high vs low doses of indole hallucinogens have functionally opposite effects on central serotonin systems, conclusions about the underlying mechanisms which mediate the behavioral effects of such drugs are also critically dependent on the exact dose used.

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