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Distinctions Between Stereotyped Sniffing and Licking in Rats with Methamphetamine and Apomorphine[1]

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SCHULZ, E. M., J. W. WRIGHT AND J. W. HARDING. *Distinctions between stereotyped sniffing and licking in rats with methamphetamine and apomorphine.* PHARMAC. BIOCHEM. BEHAV. 15(3) 521–523, 1981.—Apomorphine-induced sniffing and licking were measured in normal rats and in rats treated chronically with methamphetamine. A dosage of 0.3 mg/kg apomorphine elicited a large, reliable pattern of sniffing and licking, but no biting. As apomorphine dosage was increased to 0.7 mg/kg in normal rats, sniffing increased whereas licking decreased. Methamphetamine treatment reduced the duration, but increased the frequency, of sniffing, and decreased the frequency of licking.

Rats Stereotypy Sniffing Licking Methamphetamine Apomorphine

REPORTS concerned with the effects of intracerebral lesions, drug injections [3,4] and pharmacological treatments [8,9], on stereotyped behaviors in rats, have tentatively identified two different types of dopamine receptors operative in amphetamine-induced sniffing and licking [5], and in apomorphine-induced biting, sniffing, head and limb movements and locomotor activity [9]. The present study was part of an investigation on the effect of continued amphetamine administration upon dopamine receptors. Apomorphine-induced sniffing, licking and biting were assessed in normal rats and in rats treated chronically with methamphetamine.

METHOD

Male, Sprague-Dawley rats, 100–120 days old, were housed individually in steel and wire cages (21×21×35 cm) in a room maintained at 21°C. A 12-hour light-dark cycle was initiated at 0600 hours. Tap water and rodent blox were provided ad lib. The behavioral observation cages were identical to home cages, except that food and water were not provided.

Dose-response relationships for apomorphine-induced behaviors were established with a group of eight normal rats. Animals were adapted to the observation cages for one hour daily three days prior to, and throughout, behavioral testing. Between 0800 and 1000 hours on testing days, rats were habituated for one-half hour to observation cages, then injected at one minute intervals with normal saline or apomorphine, and returned to the observation cages. Apomorphine-hydrochloride was prepared in normal saline twenty minutes before injection, and was administered subcutaneously into the flank in a 1.0 ml/kg volume. Apomorphine dosages were randomized and administered at two to four day intervals. Methamphetamine-treated rats were tested with apomorphine the day following administration of 5.0 mg/kg methamphetamine-hydrochloride twice daily for 10 days and 10.0 mg/kg twice daily for an additional 10 days. Methamphetamine in normal saline was injected intraperitonially in a 1.0 ml/kg volume.

One minute observations were made by an observer blind to the treatment condition on each animal at 8, 16, 30, 45 and 60 minutes postinjection. Sniffing, licking and biting were recorded on an Easterline-Angus multievent recorder with paper speed set at one inch per minute. Pen trace lengths for each behavior were converted to seconds/60-seconds as frequencies of display within times post-injection, and to seconds/5×60-seconds as total frequencies of display within dosage levels. Frequencies were analyzed by one-way analysis of variance for repeated measures and equal N's, and by Biomed analysis of variance for repeated measures and unequal N's. Posthoc tests of significance were made with the Newman-Keuls distribution.

RESULTS AND DISCUSSION

Sniffing and licking frequencies were related to apomorphine dosage, $F(7,49)=65.0$ and 7.4 respectively, p's<0.001, and to time postinjection, $F(4,28)=67.2$ and 7.6 respectively,

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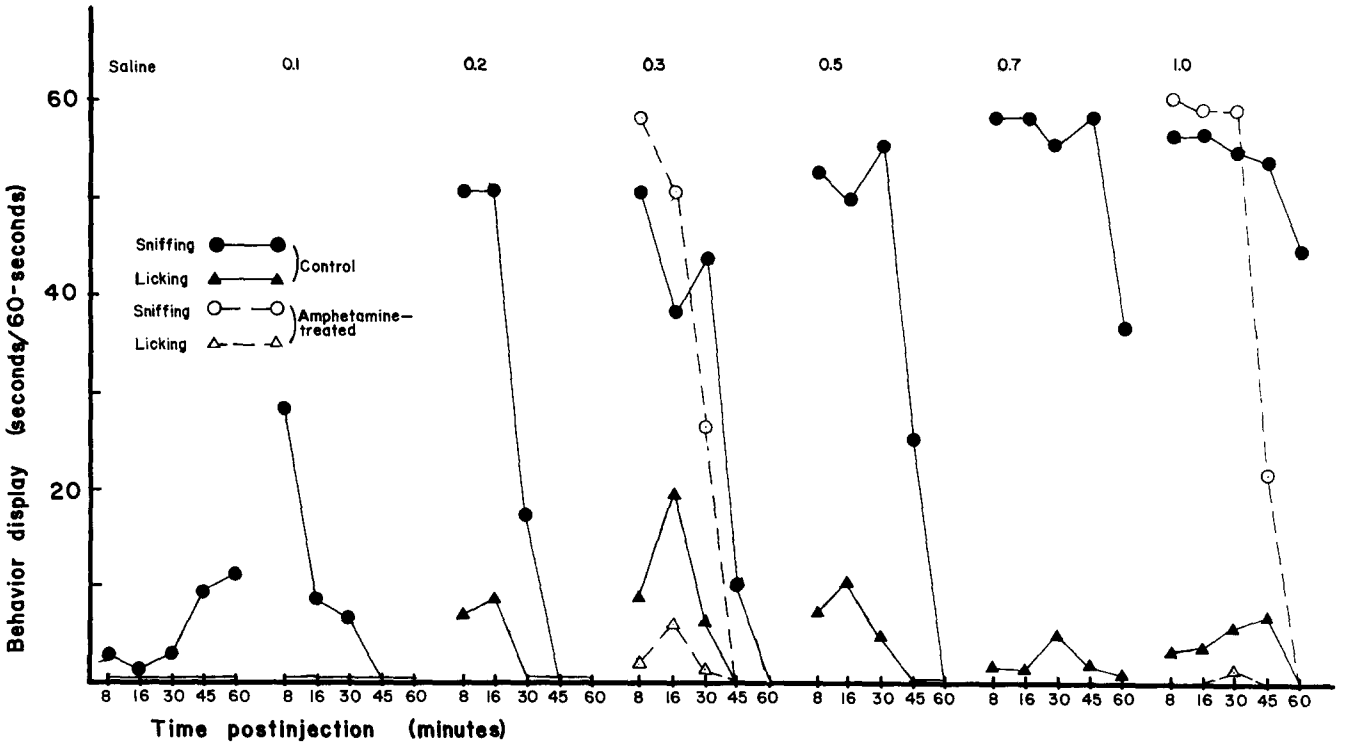

FIG. 1. Apomorphine-induced sniffing and licking: mean frequencies (sec/60-sec) based on n animals are plotted. Controls: n=8 with 0, 0.1, 0.2, 0.5, 0.7, and 1.0 mg/kg apomorphine; n=48 with 0.3 mg/kg apomorphine. Methamphetamine treated: n=8 with 0.3 mg/kg, n=6 with 1.0 mg/kg apomorphine. Standard errors of means (sniffing) at 8, 16, and 30 minutes postinjection of 1.0 mg/kg apomorphine were 0.8 seconds in methamphetamine treated rats. Standard errors increased as frequencies decreased. Pooled standard errors: Controls: n=8, 4.23 sec (sniffing) and 2.3 sec (licking). Controls: n=48, 2.04 sec (sniffing) and 1.21 sec (licking). Methamphetamine treated: n=8, 5.0 sec (sniffing) and 3.0 sec (licking). Methamphetamine treated: n=6, 4.9 sec (sniffing) and 0.5 sec (licking).

p's<0.001. An interaction between dosages and time postinjection was also present for both behaviors, $F(28,196)=8.4$ and 3.4 respectively, $p<0.001$. Biting was not displayed with the dosages of apomorphine used in this study.

Apomorphine-induced sniffing and licking in normal and methamphetamine treated rats are shown in Fig. 1. As apomorphine dosage was increased from 0.3 to 0.7 mg/kg in normal rats, sniffing increased from 144 to 266 seconds/5×60-seconds ($p<0.01$), while licking decreased from 34 to 10 seconds/5×60-seconds ($p<0.01$). This observation appears to be in contrast to an established trend in which 'gnawing, biting and licking,' when taken as a single behavior, replaces sniffing as apomorphine dosage exceeds 1.0 mg/kg [3]. However, licking became more frequent (19 seconds/5×60-seconds) while sniffing remained constant (265 seconds/5×60-seconds) as apomorphine dosage was increased from 0.7 to 1.0 mg/kg. It is also apparent from Fig. 1 that as apomorphine dosage is increased, maximum licking behavior occurs later in the postinjection period.

These trends did not appear to result from behavioral tolerance or sensitization due to repeated testings with low doses of apomorphine. Posthoc examination of the injection schedule revealed that 0.5 mg/kg apomorphine was injected before, and 1.0 mg/kg was injected after, 0.3 mg/kg apomorphine. Based on within animal comparisons (n=8), 0.3 mg/kg elicited more licking, but less sniffing than the other two dosages (p's<0.05, all comparisons except 0.5 mg/kg-sniffing). Separate groups of methamphetamine treated animals also showed a lower frequency of licking with 1.0, compared to 0.3, mg/kg apomorphine. And in a previous study [12], Sprague-Dawley rats responded with equal amounts of stereotypy to three consecutive daily injections of 0.5 mg/kg apomorphine.

Methamphetamine-treatment increased sniffing by 10 seconds/60-seconds during the first 16 minutes postinjection of 0.3 mg/kg apomorphine ($p<0.01$), and by 3.6 seconds/60-seconds during the first 30 minutes postinjection of 1.0 mg/kg apomorphine ($p<0.01$). Licking, on the other hand, was reduced to the same extent at these times and dosages (p's<0.01). Methamphetamine-treated rats also showed an earlier offset of sniffing. Normal animals, after injection of 0.3 and 1.0 mg/kg apomorphine displayed sniffing for 45 and 60 minutes respectively. Methamphetamine-treated rats displayed sniffing for only 30 and 45 minutes at these dosages. Saline-injected controls for methamphetamine treatment were not included in this study. However, previous studies have established that daily saline injections do not alter the dose-response relationships of apomorphine-induced stereotypy in Sprague-Dawley rats [12], nor the apomorphine-stereotypy thresholds in guinea pigs [7].

The effects of methamphetamine treatment reported here are comparable to studies with d-amphetamine. Methamphetamine is metabolized to a large extent in rats to form amphetamine [1]. Mass spectrographic analysis shows only hydroxylated metabolites of amphetamine in brain tissues

from methamphetamine pretreated rats [2]. Methamphetamine and amphetamine are equivalent in producing stereotypy in rats [11], and are identical also in eliciting 'sniffing, licking and biting' in mice when administered acutely or chronically at several dosages [10].

Our results are in close agreement with a recent study in which Sprague-Dawley rats showed increased sniffing and decreased licking when injected repeatedly with d-amphetamine [5]. While d-amphetamine has predominantly presynaptic actions, apomorphine has characteristics of a direct dopamine receptor agonist [6]. The present results therefore provide more direct support for the possibility that increased sniffing and decreased licking in chronically meth-, or d-amphetamine-treated rats result from dopamine receptor changes rather than presynaptic metabolism alterations. A possible exception may be in the mechanisms for a briefer duration of apomorphine-induced sniffing and a prolonged duration of d-amphetamine-induced sniffing [5] in chronically treated animals.

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