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BRIEF COMMUNICATION

Delayed Appearance of Facial Tics Following Chronic Fluphenazine Administration to Guinea Pigs

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WEINSTEIN, D., R. E. SEE AND G. ELLISON. *Delayed appearance of facial tics following chronic fluphenazine administration to guinea pigs*. PHARMACOL BIOCHEM BEHAV **32**(4) 1057–1060, 1989.—Guinea pigs were administered chronic fluphenazine decanoate for 11 months and oral movements (OMs) were periodically observed using several different paradigms. Shortly after the initiation of neuroleptic treatment, increased OMs were seen in the drugged animals, but these did not persist and may have reflected a decreased fear (freezing) reaction in the tranquilized animals, being correlated with increased locomotion in open field. After 7 months of neuroleptics, twitch-like movements of the orofacial region were observed in the drugged animals; these dyskinetic movements were enhanced by administration of d-amphetamine. These twitch-like movements appear to be a better model of tardive dyskinesia in the guinea pig than the initially observed and normal-appearing OMs.

Tardive dyskinesia Chronic neuroleptics Fluphenazine Guinea pigs

APPROXIMATELY 20–30% (8) of patients receiving chronic neuroleptics develop tardive dyskinesia (TD) as manifested by repetitive, involuntary movements of the orofacial region or, in some cases, of the trunk, arms, and legs. The causes and treatment are not well understood (4, 10, 22). A variety of species, from rats to primates, have been used in previous attempts to model human TD, with varying degrees of success (2, 6–8). The present study was designed to examine the efficacy of guinea pigs as an animal model for TD. Guinea pigs might be expected to be an especially promising species, with a high frequency of spontaneous oral activity and a highly articulated facial region. While guinea pigs have rarely been used in studies which attempt to demonstrate the appearance of spontaneous TD-like OMs following prolonged neuroleptic treatment as are seen in rats (5, 18, 21), they do show substantial DA supersensitivity following chronic neuroleptics (11, 12, 24). The animals in the present study received chronic fluphenazine decanoate (FLU) for 11 months, and throughout this time OM activity was recorded by either a human observer, by a computerized observation apparatus, or both.

METHOD

Subjects

Female, albino guinea pigs (N=39) (Hartly strain), initial weight averaging 543 g, were individually housed and maintained on a 12-hr light/dark cycle. The animals were initially divided into three equal groups: a "High" dosage group, which received an injection of 2.8 mg/kg FLU once every two weeks; a "Low" dosage group (similar injections of 0.7 mg/kg); and a "control" group. The FLU was diluted in 0.5 ml sesame oil vehicle; the control group received a similar injection of sesame oil. During the course of the nearly year-long experiment, 5 to 7 animals (from each experimental group) died, most of these in a epidemic of respiratory infections during the middle of the experiment, so that near the end of the experiment the sample size was "Control"=8, "Low"=6, and "High"=7. Because of this decreased N, and because the data at this time were not indicating TD-like OMs in either drug group, following 9 months of chronic treatment the FLU dose was increased to attain a level of 1 mg/kg/day for both drug-treated groups (15 mg/kg injected twice monthly during the 9th and 10th month). This dose has been found to induce spontaneous OMs in rats (20).

Behavioral Observations

These were conducted at least once each month throughout the experiment, with most occurring midway between injections. The

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procedures used for these observations were like those previously described for rats: placing the animals in circular restraint tubes for 5-min testing sessions, a human observer viewing close-up television images, and employing the computerized video detection device previously described for rats (3, 19, 20) and adapted for guinea pigs. Prior to testing, each animal was removed from its cage, fluorescent dots were painted on its upper and lower jaw, and it was placed in a tube similar to that described for rats (19) but larger (5″ × 12″). The animal was tested in a soundproof chamber, positioned facing towards a video camera with a UV filter attached. A human observer pressed keys corresponding to various types of oral and head movements, and a computer circuit also stored the position of the fluorescent dots from the camera; these were subsequently analyzed for vertical openings and closings of the mouth using programs described previously (3).

The location for testing was changed after the third month, when it became apparent that testing the guinea pigs by placing them in a novel room was not effective, since the skittish animals then "froze" and became completely motionless. To minimize this in subsequent testing, all testing was moved to the animals' home environment room where better results were obtained.

Open-Field Observations

During the first month of the study, 5-min open-field observations were also conducted one day prior to and two days following the initial two drug injections in a large cylindrical apparatus with a 4-foot diameter and white lines dividing the floor into 30 squares (5″ × 5″). The pig was placed in the center and each time the animal stepped into a new square, a locomotion count was recorded.

Dome Observations

At 11 months (after one month in which no drugs were administered), all animals were tested twice, 20 min after a single injection of either saline or D-amphetamine sulfate (D-amp; 0.3 mg/kg) and were observed 20 min thereafter. For this test a less obtrusive apparatus was constructed consisting of a transparent plastic dome, placed on top of a large mirror (for increased visibility of the mouth). In these tests, which were again conducted in the same room as the animals were housed, each animal was observed for a 5-min period. High and low drug groups were combined and data were analyzed using a two-way analysis of variance.

RESULTS

At month two the computerized device indicated a significantly elevated number of small-amplitude OMs in the drugged animals (smallest amplitude OMs were "Controls": 187 ± 26; "Low": 205 ± 25; "High": 243 ± 17; next highest amplitudes: "Controls": 16 ± 3; "Low": 24 ± 8; "High": 21 ± 6; $p<0.05$, trend tests). However these initial behavioral observations in the testing tube did not seem meaningful, for during these first few months, behavioral measures were extremely low in all animals due to the fact that all guinea pigs demonstrated a pronounced fear reaction, resulting in an almost complete immobilization of the animals in novel testing conditions, such as in the restraint tube. Further evidence that these effects were due largely to alterations in a "freezing" response was the fact that, when tested at one month in the open field, the drugged animals were significantly more active than the controls 2 days after injection [total frequency of locomotion counts: "Control" 14.1 ± 5.6, "High" 65.5 ± 12.2, "Low" 43.6 ± 10.7, $F(2,35)=6.4$, $p<0.005$].

After 3 months of chronic neuroleptics, when the testing apparatus was moved to the home cage, all animals showed more activity. Again the drug-treated pigs showed significantly increased head and OMs in the tube test both prior to and following drug injections, however, since all behaviors were similarly affected, this increased activity may have again been due to tranquilization and sedation of the fear response. During the fourth month of treatment, computerized data again showed vertical OMs to be significantly higher in FLU animals than in controls [computer-scored movements/min was "Control" 98 ± 12.1, "High" 136 ± 11.7, "Low" 125 ± 15.9, $F(1,30)=4.99$, $p<0.05$]. However, this increase in vertical OMs seen in the drug-treated animals then gradually decreased during months 5 to 8, human observer scores declining from an average in the drugged animals of 8.6 ± 1.5 at month 2, to 1.1 ± 0.3 at month 6, and eventually disappeared entirely for the remainder of the study. In contrast, lateral OMs scores increased steadily as the study progressed in all three groups, apparently demonstrating marked habituation to the testing apparatus. Lateral OMs have the appearance of normal chewing behavior.

Following seven months of chronic FLU administration, it was first noticed that small tic-like OMs were present in the FLU animals. These appeared as unilateral, rapid, and sometimes repetitive dyskinetic contractions of the muscles surrounding the lips. These twitch-like responses, which developed in about half of the animals, had a characteristic form and location on the muzzle which was different for each individual pig, but they were predominantly on the left side of the face. By 10 months, human observations indicated that these twitches were greater in the neuroleptic group, $F(1,27)=4.48$, $p<0.05$, than in the controls (where they were rarely seen). Because they appeared at different places on the muzzle in different animals, the computerized device could not be used to measure them. Accordingly, the behavioral testing conditions were altered so as to better permit their quantification by the observer.

When tested at 11 months (1 month after final FLU injection), it was found that while these twitches were completely absent in saline-injected controls, they were significantly elevated in the FLU-treated animals, and increased even more following a low challenge dose of D-amp, $F(1,17)=8.89$, $p<0.01$ (Fig. 1). Lateral OMs ("chewing") actually decreased in the FLU animals upon D-amp injection, whereas both lateral "chewing" OMs and head movements were elevated in the controls following the D-amp injection [Lateral: $F(1,17)=5.5$, $p<0.03$; Head: $F(1,17)=3.57$, $p<0.07$ (Fig. 1)]. The incidence of vertical OMs was not significantly changed by D-amp injection in either group.

DISCUSSION

These results indicate that guinea pigs provide a very different rodent animal model of TD compared to previous reports using rats (21). In their home cages, guinea pigs exhibit a relatively high baseline of OMs as part of their normal behavior, suggesting that they might serve as an ideal rodent model for TD research. However, when placed in a novel testing cage, these animals have a pronounced fear reaction which results in the inhibition of all movements, including oral activity. This problem was attenuated in the present studies only by prolonged habituation to the testing apparatus and by testing the animals in the same room in which they were housed. When these precautions were taken, it was found that the increases in vertical OMs observed in the drug-treated animals showed a pattern of response expected of TD (exaggerated in the drugged groups, and in a dose-dependent fashion). However, these movements did not show a second characteristic of TD, for they did not persist in the drug-treated animals, for following 10 months of chronic neuroleptic treatment and extensive habituation to the testing cages all of the animals tended only to show lateral, chewing-like oral activity.

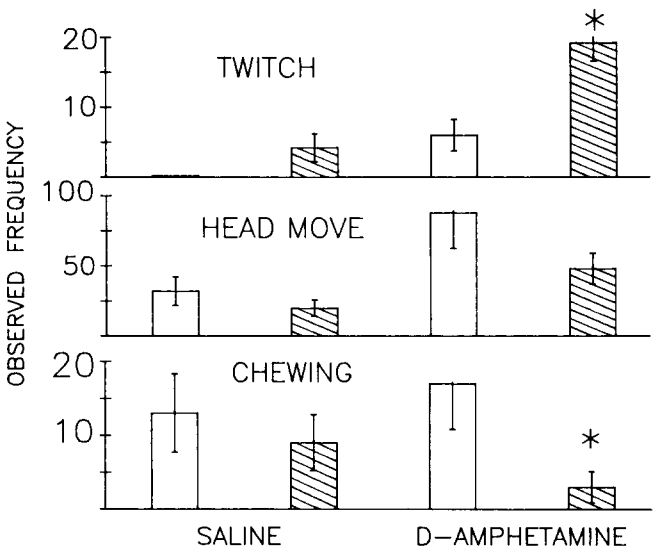

FIG. 1. Mean number of "twitch-like" oral movements, head movements, and lateral ("chewing") oral movements following saline or D-amp (0.3 mg/kg) injection. These data are total number observed during the 20-min test. Open bars: controls (±S.E.M.); hatched bars: fluphenazine-treated animals.

While the spontaneous, vertical OMs dissipated entirely over time, suggesting they were similar to the artifactual OMs sometimes observed in rats given neuroleptics (13), they were eventually replaced by an abnormal, twitch-like behavior which appeared dyskinetic in nature. Because TD in humans is characterized by both its slow appearance and selectivity for a certain subset of treated patients (9), of the various behaviors observed in this study, the twitching behavior exhibited by the FLU-treated animals most resembled that of human TD, both because of its dyskinetic and individualistic appearance and in its delayed onset. In addition, an exacerbation of twitching activity was seen following a challenge dose of the DA agonist D-amp. Increases in dyskinetic activity following DA agonists is a well-established phenomenon in humans with "classical" TD (14), as are increases in stereotyped OMs in rodents following prolonged neuroleptic exposure (15). Other behaviors more indicative of overall activity levels, such as lateral OMs and head movements, were elevated in the control group following the D-amp injection. This pattern of behavioral alterations demonstrates that the increase in twitching behavior was not simply due to an increase in overall activity.

A controversy currently exists as to whether or not TD-like OMs can be induced in rodents through chronic neuroleptic treatment (13, 16, 17, 23). One of the problems with currently available rat models is that virtually all measure OMs which are normal in appearance. The results from the present study are highly unique in that they demonstrate a late-developing abnormality in the oral-region of neuroleptic-treated guinea pigs which is completely absent in saline-injected controls and which is dyskinetic in appearance. Even more importantly, they show a distinctive feature of human TD movements (1): a predominance on the left side of the face. These twitch-like behaviors may, therefore, be more similar to the types of dyskinetic movements observed in human TD than those which occur in rats.

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