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Failure to Find Electrophysiological Correlates of Chronic Neuroleptic-Induced Oral Dyskinesias in Cats: Somatosensory and Substantia Nigra Evoked Potentials, Electroencephalogram, and Caudate Spindles

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GLASSMAN, R. B., H. N. GLASSMAN AND B. M. BALTRUS *Failure to find electrophysiological correlates of chronic neuroleptic-induced oral dyskinesias in cats: Somatosensory and substantia nigra evoked potentials, electroencephalogram, and caudate spindles.* PHARMAC. BIOCHEM. BEHAV. 17(5) 1061–1066, 1982.—Each of nine cats was prepared with 30 chronically implanted stainless steel gross electrodes in cortex, basal ganglia and other brain structures. Measurements were taken for 0.5–11.5 months of baseline, chronic daily administration of chlorpromazine, and withdrawal. In most cases there was also a second cycle of drug administration and withdrawal. Although we observed dramatic, persistent increases in licking behavior, suggestive of tardive dyskinesia, consistent correlated patterns were not observed in somatosensory or substantia nigra evoked potentials, electroencephalogram, or spindling evoked in cortex by caudate stimulation.

Electroencephalogram Evoked potentials Neuroleptics Oral dyskinesia Tardive dyskinesia

MANY schizophrenic patients who have received chronic administration of neuroleptics for months or years develop tardive dyskinesia, a syndrome of choreiform movements whose symptoms include licking, chewing, and other movements of the mouth. Although investigators have tended to focus hypotheses about the causes of tardive dyskinesia on known or conjectured changes in striatal dopamine synapses, there is sufficient uncertainty to warrant exploratory studies to seek other relevant factors [1, 2, 6].

Our approach was to use a chronically implanted gross electrode technique to repeatedly observe activity in many brain regions over long periods of time, in awake animals. After preparing each of nine cats with thirty cortical and deep electrodes, we recorded the following on a regular schedule before, during and after chronic administration of chlorpromazine: (1) behaviors which occurred spontaneously, (2) potentials evoked by peripheral somatosensory stimulation, (3) potentials evoked in basal ganglia by substantia nigra stimulation, (4) spontaneous electroencephalographic activity, (5) EEG spindles in cortex evoked by stimulation of points in the basal ganglia [5]. Additional occasional measures were taken of evoked potentials following stimulation of selected points.

We report here briefly on the clear behavioral effect that was observed and on our failure to discern a correlation of this effect with the electrophysiological measures.

METHOD

Surgery

Arrays of 30 monopolar gross electrodes were implanted into nine adult cats using pentobarbital anesthesia. The cats were divided into three groups to accommodate all targeted areas. Histologically verified placements were in each of the following regions in at least two cats. (a) Cortex: gyrus proreus, anterior sigmoid gyrus, orbital gyrus, anterior sylvian gyrus; (b) basal ganglia: caudate nucleus, globus pallidus, putamen, claustrum, entopeduncular nucleus; (c) thalamus: ventroanterior, ventrolateral, ventroposterolateral, ventroposteromedial, central lateral, mediodorsal; (d) brainstem: red nucleus, reticular tegmentum, substantia nigra; (e) limbic regions: olfactory tract, nucleus accumbens, amygdala, lateral hypothalamus.

To reduce damage to the brain, arrays of subcortical electrodes were planned so that no two electrodes were closer than 2.5 mm apart, with the exception of contiguous pairs whose tips were positioned at different heights. Electrodes

Behavioral Measures

The cat was carried from its home cage and was placed in a wire cage (90×53×60 cm high) in an adjoining room. The animal was then observed for five minutes and counts were made of all instances of licking, grooming, and shaking of the paw or shaking of other parts of the body. Additional, detailed qualitative notes were also taken. Two observers alternated carrying out behavioral observations in the morning, before the other scheduled tests.

Physiological Measures

Evoked potentials—peripheral stimulation. Evoked potentials (EPs), usually triggered at a frequency of one/sec, were recorded monopolarly against an indifferent electrode in the bone over the frontal sinus, using a sweep speed of 10 msec/cm; three overlapping, consecutive sweeps were viewed and photographed at the same time. Sweeps were recorded at each point whether or not it had shown an EP during previous sessions. The technique of peripheral somatosensory stimulation has been described previously [4]. Briefly, shocks 5 mA, 0.1 msec duration, originating from an electrically isolated constant current stimulator were delivered to two electrodes, one taped to the forelimb pad and the other to the shaved lateral surface of the elbow. All nine cats were tested in this manner.

Evoked potentials—central stimulation. An analogous method was used to record EPs that followed central stimulations in four of the cats. For negative, monopolar substantia nigra stimulation the stimulating indifferent electrode was a 15 cm stainless steel suture wire implanted subcutaneously near the margin of the electrode rig. Recordings were taken only from electrodes in the basal ganglia. Additional occasional sessions were carried out with some animals, in which sweeps were recorded at selected points using stimulation of electrodes in basal ganglia, thalamus, reticular formation, hypothalamus, or amygdala. Stimulus duration was always 0.1 msec and stimulus amplitude was 1 mA unless the cat's behavior during the earliest sessions suggested that this intensity was aversive; in those cases 0.5 mA was used throughout the experiment. When central points were stimulated, a complete set of recordings was made at each of two sweep speeds, 0.5 msec/cm and 10 msec/cm, in order to see both early and late components of the EPs. Maps of the daily evoked potential records were pasted up for each cat.

Spontaneous electroencephalographic activity and evoked spindling. Recordings of spontaneous EEG activity, at least 20 seconds in duration, were taken from each electrode in all animals. With four of the animals, additional recordings were carried out at five cortical points, using stimulation of electrodes in basal ganglia to evoke spindles in the cortex. For this purpose, single, negative monopolar pulses (1 ma, 0.1 msec duration) were delivered to the stimulating points at intervals of one every two seconds.

In order to restrain the animal comfortably during the recording of EEG activity or of potentials evoked by central stimulation, the cat wore a leather collar that was attached to an aluminum rod; the other end of the rod was attached to the table using additional rods and adjustable joints. For recording peripheral somatosensory EPs the cat was laced into an adjustable sling so that the limbs were suspended over the table. After several habituation sessions the animals remained calm under this restraint, for the 15 to 30 minute session.

Scheduling

Behavioral and physiological measures were taken more frequently when drug treatment was initiated or withdrawn than during the long periods of chronic treatment. Following initiation of drug, a change in dosage, or withdrawal, behavioral measurements were taken five to seven times a week for two weeks, and physiological measurements were carried out at one day, two days, four days, and one week. Thereafter, behavioral measurements were taken three times a week and physiological measurements at intervals of every two weeks until the next change in chronic treatment conditions.

Following surgical implantation of electrodes, drug treatment was begun after a three to four month baseline period with the first four animals tested and after six to 9.5 months of baseline with the remaining five animals. During the baseline periods at least thirteen behavioral sessions were carried out with each animal. There were between eight and nineteen baseline sessions of EEG and of peripheral EP recording in different cases, and there were at least six baseline sessions of substantia nigra EP recordings.

Chronic drug administration periods for our earlier observations were shorter because we wanted to learn what effects might result from periods of as short as a month. For the later observations we used longer drug administration periods both to more closely simulate conditions used with patients and to try to obtain clearer physiological effects. Overall, chlorpromazine was administered for periods of 1 to 11.5 months, followed by withdrawal periods of 0.5 to 3 months in different animals or with repeated treatments in the same animals. To test for possible cumulative effects, some animals underwent up to three drug and withdrawal cycles, within the above guidelines. Chlorpromazine, in pill form, was administered orally once a day after measurements for that day had been taken. The doses, set at between 25 and 50 mg/day for different cats, appeared to be as much as the individual animals could tolerate while still maintaining food and water intake. The animals' weights ranged from 2.2 to 3.6 kg and the mg/kg dosages for each cat therefore worked out as follows: R1—11; R3—15; R4—15; R8—15; R6—16; R7—16; SP1—15; SP9—21; BH2—17. Cats were housed individually so that food and water intake could be monitored. They were weighed every day and the margin around the electrode rig was cleaned with a soap solution and dried.

Histological Verification

After being anesthetized deeply with an overdose of pentobarbital, animals were perfused intracardially with normal saline followed by 10 percent Formalin. In most cases perfusion was preceded by a procedure in which a small amount of current was passed at each electrode; the Formalin solution contained potassium ferrocyanide, which served to mark the position of the electrode tip with a blue dot. The brains were embedded in celloidin and were sectioned at 50 microns. Sections were examined at intervals of 0.25 mm. Alternate sections were stained using cresyl violet and by the Weil method. All of the electrode locations discussed here have been verified histologically.

RESULTS

Chronic drug treatment was associated with a clear behavioral effect but not with consistent effects in our electrophysiological measures. Drug treatment or withdrawal

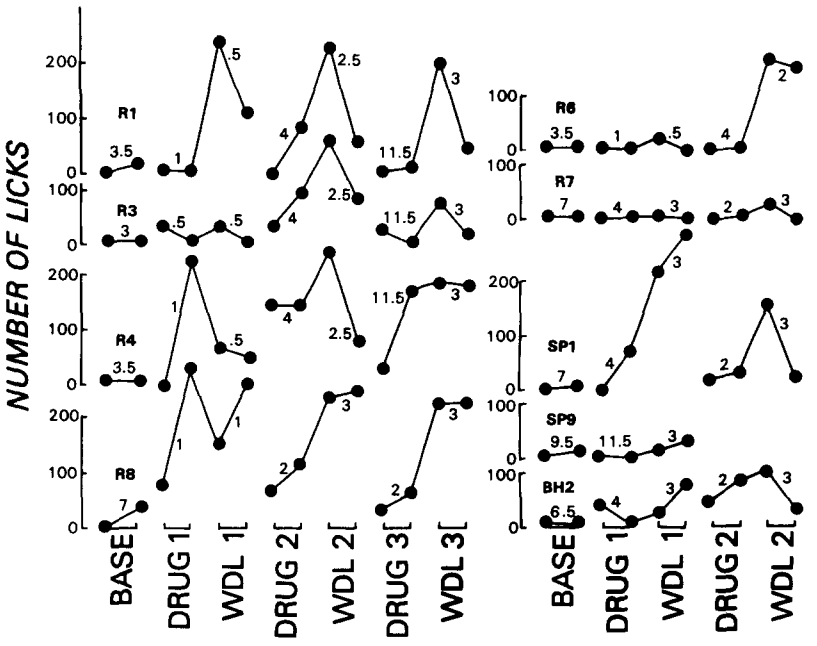

FIG. 1. Number of licking movements made in five-minute observation sessions at the beginning and end of baseline and of each drug administration and withdrawal period. Each dot represents an average over 5–7 observation sessions that were carried out during one week (at the start of a drug or withdrawal period) or two weeks (at the end of drug or withdrawal periods; see "Scheduling" in the description of procedures). The numbers adjoining the curves indicate how many months that animal spent under the particular condition. Additional data obtained during these periods showed fluctuations but were generally consistent with the picture presented here. With some animals, baseline measurements and measurements after the last withdrawal were carried out for a longer time with respect to behavior than with the physiological measures shown in Figs. 2, 3, and 4; therefore these first and last month-indicating numbers in Fig. 1 do not match those in the other figures. For readability, lines join the points for a given drug and withdrawal cycle and a space separates each such set of treatment intervals from the next. Points indicating the licking scores obtained early in withdrawal represent measurements taken during the week immediately following the drug period. Similarly, the early drug scores shown are those for the week immediately following the preceding baseline or withdrawal period.

was accompanied by an increase in the number of licking movements in every animal, in at least one of the drug-withdrawal cycles. In some cases the increase was dramatic—over 200 licks in a five-minute observation period. Figure 1 illustrates the early and late scores for each baseline, drug, and withdrawal period in each animal. Most licks were integrated into grooming movements but many appeared in vacuo. Increased paw or body shakes occurred with some animals in addition to increased licking; in three animals "fly-chasing" movements of the skin of the back were observed for the first time on drug and were greatly increased on withdrawal. Figure 1 also indicates the number of months (from 0.5 to 11.5) spent by each animal in each drug and withdrawal period. Comparison of the individual cases reveals no consistent effect of duration of treatment and no clear tendency toward a cumulative effect of successive treatments; we cannot account for the variations among individuals. In almost all cats, the incidence of licking remained higher at the end of withdrawal than it had been during baseline.

Somatosensory evoked potentials, substantia nigra evoked potentials, and caudate-evoked cortical spindling data for the early and late portions of each baseline, drug, and withdrawal period are shown in Figs. 2, 3, and 4. These figures give a sense of the range of responsiveness of each animal and of the variation among animals. We were unable to discern any consistent pattern on examination and re-examination of the data shown in these figures or of the much larger body of data from all the measurements.

Only points that had shown evoked potentials during baseline showed them also during drug or withdrawal; that is, no new potentials developed at other electrode sites. Figures 2 and 3 represent potentials that met a criterion of 0.16 mV during baseline (2 mm on the photographed records). The point showing the largest baseline potential in each animal is the one that is graphed; the appended vertical lines

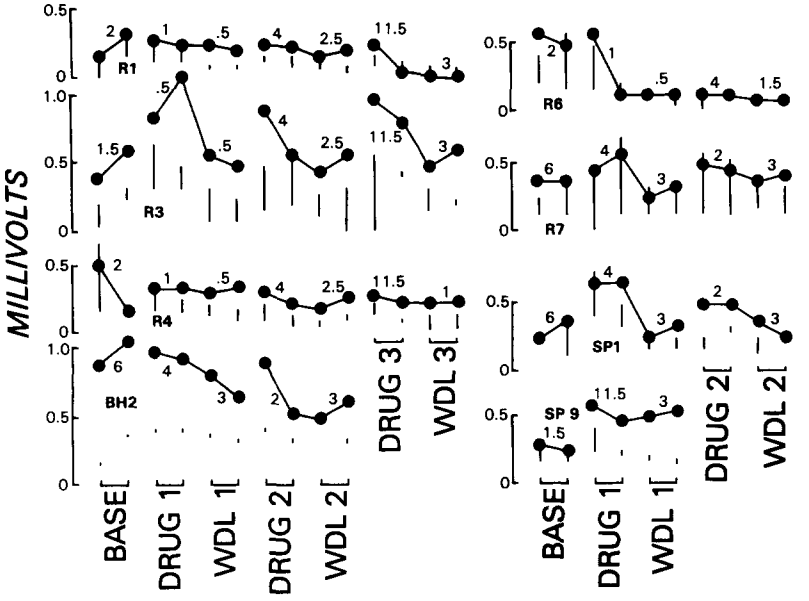

FIG. 2. Somatosensory evoked potential magnitude observed during an early baseline day and on the final baseline day, and on the second and last day of each drug and withdrawal period. For each animal, the dot represents the point that showed the largest magnitude potential during baseline, while the underlying vertical lines represent the ranges of magnitude observed at 1–5 other responsive points. In all cases but one all responsive electrodes were in cortex (in R1 the vertical line represents an electrode in globus pallidus and one in ventroposterolateral thalamus, as well as one additional cortical point). Other details of graph layout are as described in the legend for Fig. 1.

were made of full-hard temper stainless steel orthodontic wire, 0.25 mm dia. and were insulated with baked on Epoxylite varnish, except for the tips, which were sharpened to a conical shape 0.5 mm long.

show the range of amplitudes at the remaining points that had met the baseline criterion.

Eight of the nine cats showed somatosensory evoked potentials meeting the baseline criterion. All such potentials were in the cortex, except for one animal (R1) in which placements in ventroposterior thalamus and globus pallidus also were responsive to the forelimb stimulation. Substantia nigra evoked potentials were sought only from six electrodes targeted in or near the basal ganglia; the graphs of Fig. 3 represent the points verified by histology to be in basal ganglia and which also met the baseline criterion of 0.16 mV. It should be noted that while vertical lines in Figs. 2 and 3 represent ranges, the vertical lines in Fig. 4 represent spindle duration for the same representative point whose amplitude is shown. Spindle frequencies, ranging from 10 to 13 Hz in different cats, remained fairly constant within animals.

While consistent patterns were not seen in the electrophysiological data, it is perhaps worth noting that six of eight cats showed increased somatosensory evoked potentials during the first drug period, and five of seven showed this change during a second drug period. With all three measures of evoked electrophysiological activity there appeared to be a tendency for an overall reduction in amplitude during the course of the experiment. Since this possible effect is not a clear one, it would be gratuitous to discuss here whether it was due to the drug or merely to time or to glial encapsulation of electrodes.

The recordings of spontaneous electroencephalographic activity were equally unilluminating. In general, initiation of the treatment with chlorpromazine was associated with EEG slowing, more evident at cortical points than elsewhere, but no long-term effects of drug or withdrawal on amplitude or frequency were evident on repeated visual inspection of the records by each of us.

We noticed no obvious effects of drug treatment or withdrawal in the occasional sets of evoked potential recordings taken during stimulation of central points other than substantia nigra.

DISCUSSION

This exploratory study was undertaken in hope of gaining new clues as to the causes of tardive dyskinesia. Although daily oral doses of chlorpromazine over a period of months led to increased incidence of licking, systematic recording at many brain loci of a number of electrophysiological phenomena failed to turn up a clear correlate of the behavioral change. The fact that it was an oral behavior that increased most with drug treatment and withdrawal, and the fact that the incidence of licking remained greater at the end of withdrawal than it had been during baseline, suggests an analogy with tardive dyskinesia as it is seen in human schizophrenic patients.

Because this study emphasized visual inspection of large

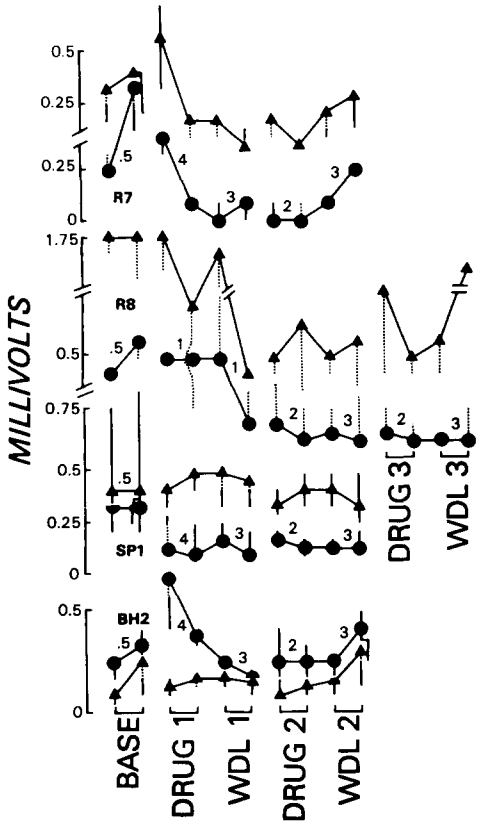

FIG. 3. Substantia nigra evoked potential magnitudes observed during an early baseline day and on the final baseline day, and on the second and last day of each drug and withdrawal period. Triangles represent the largest peak-to-peak EP seen within 5 msec (fast sweep speed) and circular dots represent the largest seen within 100 msec (slower sweep speed). In each case, the point graphed is the one that had shown the largest baseline longer-latency EP. Solid vertical lines represent the range of EPs at the other responsive electrodes in the basal ganglia. For legibility these are connected to the graph of the corresponding largest EP by a dashed line. If only one other electrode was responsive, only a dashed line is shown and its end point indicates the EP magnitude at that other electrode position. With cat R7 the largest EP came from a point between globus pallidus and putamen; the vertical line indicates EPs from three points: caudate, caudate-accumbens, and putamen-globus pallidus. Corresponding information for the other three cats is as follows. R8, largest: claustrum; one other: claustrum-cortex. SP1, largest: putamen; three others: caudate, globus pallidus-entopeduncular, caudate-corpus callosum. BH2, largest: entopeduncular nucleus; two others: caudate, putamen-globus pallidus. In the curve for the short latency EP of R8, points below the broken line represent magnitudes on the part of the ordinate below the broken ordinate line. In the graph for R7, the ordinate is broken to more clearly separate the short and long latency EP curves. Other details of graph layout as described in the legend for Fig. 1.

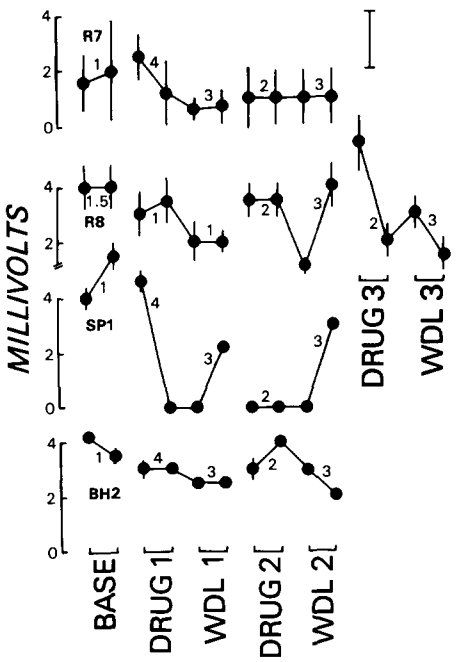

FIG. 4. Caudate spindling during an early baseline day and on the final baseline day, and on the second and the last day of each drug and withdrawal period. Dots represent the amplitude at the middle of a spindle recorded from a point in frontal cortex immediately following stimulation of a point in caudate nucleus. For cats R7, R8, and SP1 the recording point shown was in gyrus proreus; for cat BH2 it was in anterior sigmoid gyrus. Vertical lines represent the duration of the same spindle (calibration: one second). As the graphs suggest, with cats SP1 and BH2 the response was so brief on most occasions that it might better be considered an evoked potential than a spindle. Magnitudes were measured from the one of four consecutive spindles, evoked at two-second intervals, that appeared on visual inspection to best represent median values of amplitude and duration. Other details of layout as described in the legend for Fig. 1.

arrays of several categories of data from a relatively small sample of subjects, in a before-and-after design, it was geared to detecting "large" effects. Although the daily oral dosages of CPZ were roughly equalized across subjects according to the criterion of maintenance of food and water ingestion, they varied across subjects in mg/kg and in duration of administration (see Method). For these reasons, and because there was variation across animals in the behavioral effect, the power to detect subtler electrophysiological alterations was low.

Other researchers have found biochemical evidence of synaptic changes after long-term treatment of animals with neuroleptics [7]. It is natural to surmise that those changes are associated with behaviorally significant electrophysiological alterations, but these were not evident in our measures. Conceivably, both behavioral and electrophysiological sequelae of chronic neuroleptic administration would be enhanced in an animal preparation that modeled schizophrenia in some limited way. For example, we have observed that rats kept chronically on chlorpromazine were more likely to show increased incidence of chewing movements if they had previously sustained damage to frontal cortex and caudate nucleus [3].

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