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Electrophysiological Correlates of Stereotyped Sniffing in Rats Injected With Apomorphine

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VANDERWOLF, C. H. AND H. SZECHTMAN. *Electrophysiological correlates of stereotyped sniffing in rats injected with apomorphine.* PHARMACOL BIOCHEM BEHAV **26**(2) 299–304, 1987.—Macroelectrodes were chronically implanted in the olfactory bulb, dorsal hippocampus, neocortex and under the mystacial pad in a group of rats. Recordings were taken during spontaneous behavior, during exploration of novel objects and odorous material, and during handling. Similar observations were made following injection of apomorphine (1.25–5 mg/kg, SC). The lower dose of apomorphine elicited a pattern of sniffing, olfactory bulb activity and vibrissal EMG which resembled closely the patterns observed in undrugged rats sniffing while in tactile contact with a novel object. However, unlike normal rats, the apomorphine-treated rats did not orient toward novel objects or odors. Apomorphine also elicited nearly continuous hippocampal rhythmical slow activity which occurred in correlation with head movements and locomotion. It is suggested that apomorphine elicits a motor pattern which resembles normal contact sniffing but which, unlike normal sniffing, is relatively impervious to control by visual and olfactory stimuli.

Apomorphine Dopamine Electrocorticogram Hippocampus Olfactory bulb Neocortex Sniffing Stereotyped behavior Snout contact

FOLLOWING treatment with dopaminomimetic drugs such as amphetamine or apomorphine, animals do not show the normal switching between different kinds of spontaneous activity but perform some behaviors over and over again, with little variation. An example of such "stereotyped behavior" [5] is the continuous snout contact with vibrissae movements shown by apomorphine-treated rats. Such rats maintain uninterrupted snout contact with environmental surfaces and appear to be sniffing continually regardless of what else they may be doing, be it climbing, walking, circling or pivoting [8]. However, it is also possible that the snout contact and vibrissae movement of apomorphine-treated rats represents a motor automatism which is not identical to normal sniffing.

The aim of the present study is to determine whether apomorphine-treated rats exhibiting snout contact show the normal electrophysiological and behavioral correlates of olfactory investigation. The results indicate that while olfactory bulb, hippocampal, and neocortical electrocorticograms resemble those of normal rats sniffing and moving about, the behavior of the drug-treated animals does not reveal normal responsiveness to external olfactory stimuli.

METHOD

Experiments were carried out in 10 adult (300–500 g) male Long-Evans rats. The rats were anesthetized with pentobarbital (60 mg/kg, IP) and had bipolar recording electrodes (stainless steel wire 125 μm in diameter and coated with Teflon except at the cross-section of the tips) implanted bilaterally in the dorsal hippocampus and unilaterally in the somatomotor neocortex according to standard techniques [1]. A single monopolar electrode was placed in the olfactory bulb and a reference electrode was fixed in the skull over the cerebellum. A separate ground electrode was placed in the skull over the frontal neocortex. In one rat a small thermister was placed in the nasal passages by means of a hole bored through the nasal bones. With the help of a trocar, electrodes consisting of 7 strands of Teflon-coated 50 μm wire (the terminal 5 mm of Teflon was stripped off) were inserted bilaterally under the mystacial pads in 4 rats.

After a recovery period of at least 3 weeks the rats were placed on a magnet-and-coil type of movement sensor platform [9]. Using an ink-writing polygraph and a storage oscilloscope, bipolar records were taken from the neocortex, hippocampus, and muscles under the mystacial pads, while monopolar records were taken from the olfactory bulb. The activity of the olfactory bulb provides a simple means of monitoring respiration [3]. An alternative means of recording respiration was provided by the temperature changes occurring in the thermister in the nasal passages of one rat (recorded by means of a DC amplifier and Wheatstone bridge circuit). Since this method gave results which were similar (in terms of frequency) to those obtained by recording from the olfactory bulb, it appears that olfactory bulb recording yields a valid measure of respiratory frequency [3].

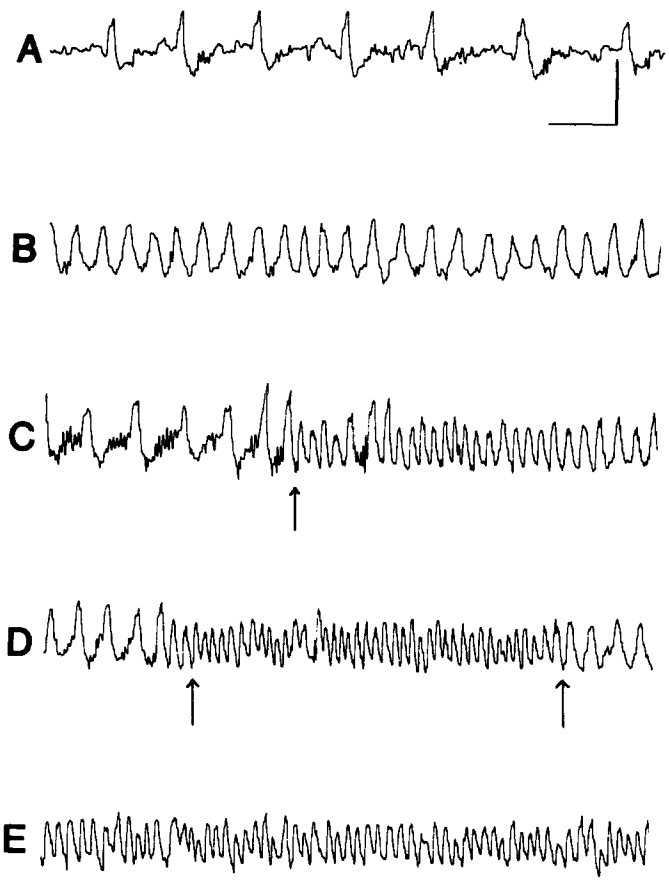

FIG. 1. Slow wave activity of rat olfactory bulb under various conditions (rat No. 1685). A, during awake immobility; B, during awake immobility; C, sniffing without snout contact, beginning at arrow; D, sniffing with snout contact with the substrate, between arrows; E, sniffing with snout contact with the substrate about 25 min after apomorphine HCl (1.25 mg/kg, SC). Cal.: 1 sec, 0.5 mV.

The occurrence of various spontaneous behaviors and the effects of handling and presentation of odors (mainly sawdust litter that had been in contact with rats or their excrement) were noted on the polygraph by means of manually operated signal markers and written notes. After 30–60 min of normal recording had been taken, apomorphine HCl (1.25 mg/kg, in an aqueous solution containing 0.1% ascorbic acid) was injected subcutaneously in the nape of the neck and observations continued as before. It has been established previously that injections of drug vehicle solutions have no effect on the functions studied here [9,10].

The polygraph records were analyzed by inspection and measurement with a clear plastic ruler. The period available for analysis during any particular condition varied according to the behavior of the rat. However, the data presented are based on a minimum of four 4-sec periods/condition/rat. The significance of changes in the measures under different conditions were assessed by means of the Wilcoxon test [7]. In addition, the measures obtained under different conditions in a single rat were treated as independent samples and the significance of any differences between conditions was assessed by a Mann-Whitney U-test.

RESULTS

Recordings in Undrugged Rats

When the rats were standing awake (eyes open, head held up) but motionless, respiration sometimes assumed a pattern of quick breaths taken at long intervals (about 1/sec, Fig. 1A) and sometimes assumed a more continuous pattern producing a roughly sinusoidal rhythm of 2–6 Hz in the olfactory bulb (Fig. 1B). The mean rate over periods of 10–100 sec ranged from 0.9–4.3 in 8 of the rats (Table 1; data from the 2 remaining rats were less complete and have been omitted). However, this rate sometimes varied widely. During periods of undisturbed immobility, two of the rats had occasional periods of apnea lasting as long as 4 sec. The rate of respiration during face-washing, body grooming and chattering of the teeth was very similar to the rate during immobility. When sniffing (defined as a rhythmical movement of the vibrissae) occurred, the respiration rate increased very reliably in all the rats, yielding a faster rhythmical waveform of slightly reduced amplitude in the olfactory bulb (Fig. 1C and D, Table 1). Sniffing was readily elicited by presentation of odorous material such as rubber corks or litter from a rat cage but the

response habituated quickly to any particular stimulus. Holding the rats in the hand or merely placing a hand on the rat's back while they stood on the platform, tended to inhibit such sniffing and often elicited struggling instead.

Bursts of electromyographic (EMG) activity could be recorded beneath the mystacial pads whenever the vibrissae moved visibly. Sometimes these bursts were quite rhythmical and appeared to be phase locked to the rhythm in the olfactory bulb (Fig. 2). At other times more prolonged nonrhythmical bursts occurred. EMG bursts also occurred during jaw movements when the vibrissae appeared to be immobile and in association with every breath when breathing was very deep and slow in a motionless rat. The latter type of activity had a much lower amplitude than the activity during sniffing. It is likely that the electrodes picked up activity from the buccinator and dilator naris muscles as well as the levator labii superioris muscle which moves the mystacial pad [2].

"Spontaneous" sniffing occurring with the snout and vibrissae in contact with a surface had a reliably higher frequency (7.5 Hz) than sniffing with the head held away from any surface (6.2 Hz, Table 1). However, in 4 rats, tests with a glass beaker filled with rat litter elicited sniffing of 7.3 ± 1.2 Hz (mean±standard deviation) when the beaker was held about 2 cm away from the snout and elicited sniffing of 7.5 ± 1.0 Hz when the rats were allowed to contact the beaker and litter with their snout and vibrissae. These values do not differ significantly. Thus, in the presence of a strong odor, sniffing without snout contact may have as high a frequency as sniffing with snout contact.

No clear correlations were observed between respiration and the patterns of electrical activity of the hippocampus and neocortex. Large amplitude irregular activity occurred in the hippocampus during immobility, face-washing and chattering of the teeth, but rhythmical slow activity (RSA) occurred during periods of head movement and walking. Sniffing often accompanied the latter behaviors. The frequency of sniffing was similar to the frequency of RSA (Table 1) but there did not appear to be any extensive tight coupling between the two rhythms. In 4 rats measurements based on 7–10 4 sec samples showed that sniffing had a lower frequency than RSA in the same time segments ($p<0.02$ in each rat, Mann-Whitney test). In the most extreme case, sniffing had a mean frequency of 5.0 Hz while the accompanying RSA had a frequency of 7.0 Hz. In the remaining 5 rats in which hippocampal activity was recorded, the mean frequencies of sniffing and RSA did not differ significantly, although the two rhythms sometimes differed substantially in frequency in single 4 sec samples. Neocortical activity, recorded in 6 rats, consisted of a low voltage fast pattern throughout most of these experiments. However, in 3 rats there were occasional occurrences of large amplitude (1–2 mV) 6–9 Hz waves that tended to occur in spindle-shaped bursts. These spindles were accompanied by a rapid tremor of the vibrissae and head and occurred only during periods of complete behavioral immobility. The respiratory rate during these spindles ranged from 2.3–2.5 Hz, much the same as during quiet respiration in the absence of the spindles.

Recordings in Rats Injected With Apomorphine

Following treatment with apomorphine, the rats became very active, moving the head and walking almost continuously for long periods. The snout remained in close contact with the surface of the platform and vibrissae movement (and correlated EMG activity that resembled the pattern seen during normal sniffing) was virtually continuous. The activity present in the olfactory bulb also resembled the pattern seen during normal sniffing with contact (Fig. 1E, Table 1).

However, despite their incessant sniffing, the rats were not responsive to odors. Presentation of rat litter, corks, etc., at the snout or 2 cm away, did not, on any occasion, elicit orientation to the stimulus, and did not change the ongoing pattern of olfactory bulb activity. The rats continued to orient to the floor of the apparatus as they did in the absence of the introduced odor.

In association with the incessant head movement and locomotion, hippocampal activity consisted of virtually continuous RSA of a frequency very similar to the frequency observed during normal head movement and locomotion (Fig. 3, Table 1). Neocortical activity consisted of a continuous low voltage fast pattern and spindle activity was no longer observed.

On rare occasions the apomorphine treated rats briefly ceased sniffing and moving about. Hippocampal activity became irregular and sharp waves occurred during these periods of immobility (Fig. 3). A similar effect could be produced by picking a rat up and holding it with the hand around the upper part of the body. The rapid pattern of respiration (mean of 7.5 Hz) was promptly replaced by a pattern of quiet respiration (mean of 4.5 Hz, Table 1). This decline in

TABLE 1

FREQUENCY (Hz) OF OLFACTORY BULB SLOW WAVES AND OF HIPPOCAMPAL RHYTHMICAL SLOW ACTIVITY (RSA) UNDER VARIOUS CONDITIONS IN 8 RATS

Condition	Mean	Standard Deviation
1. Olfactory bulb activity during immobility and quiet breathing	3.3*	1.2
2. Olfactory bulb activity during sniffing, no snout contact	6.2†	0.8
3. Olfactory bulb activity during sniffing with snout contact	7.5	0.6
4. Olfactory bulb activity during sniffing, RSA present	6.9	1.0
5. Hippocampal RSA frequency during sniffing episodes in No. 4	7.4	0.2
6. Olfactory bulb activity during sniffing after apomorphine (1.25 mg/kg)	7.5	1.0
7. Hippocampal RSA frequency during sniffing episodes in No. 6	7.7	0.4
8. Olfactory bulb activity after apomorphine, rat held in hand away from platform	4.5‡	1.2
9. Olfactory bulb activity during sniffing, after apomorphine, rat's hindquarters held up off platform, forequarters on platform	7.2	0.9

*Significantly different from each condition termed "sniffing" $p<0.01$, Wilcoxon test; †significantly different from sniffing with snout contact, $p<0.01$, Wilcoxon test; ‡significantly different from sniffing after apomorphine (Condition No. 6), $p<0.01$, Wilcoxon test.

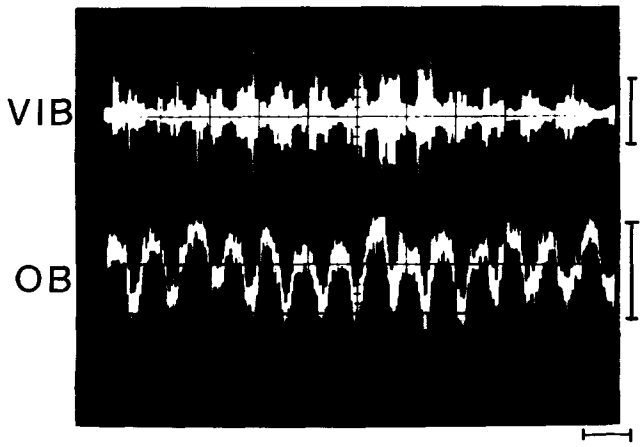

FIG. 2. EMG activity beneath the mystacial pad and slow wave activity of the olfactory bulb during sniffing in a rat. VIB, vibrissae; OB, olfactory bulb. EMG band pass 30–10,000 Hz, OB band pass, 1–10,000 Hz. Cal.: 0.5 mV, 200 msec.

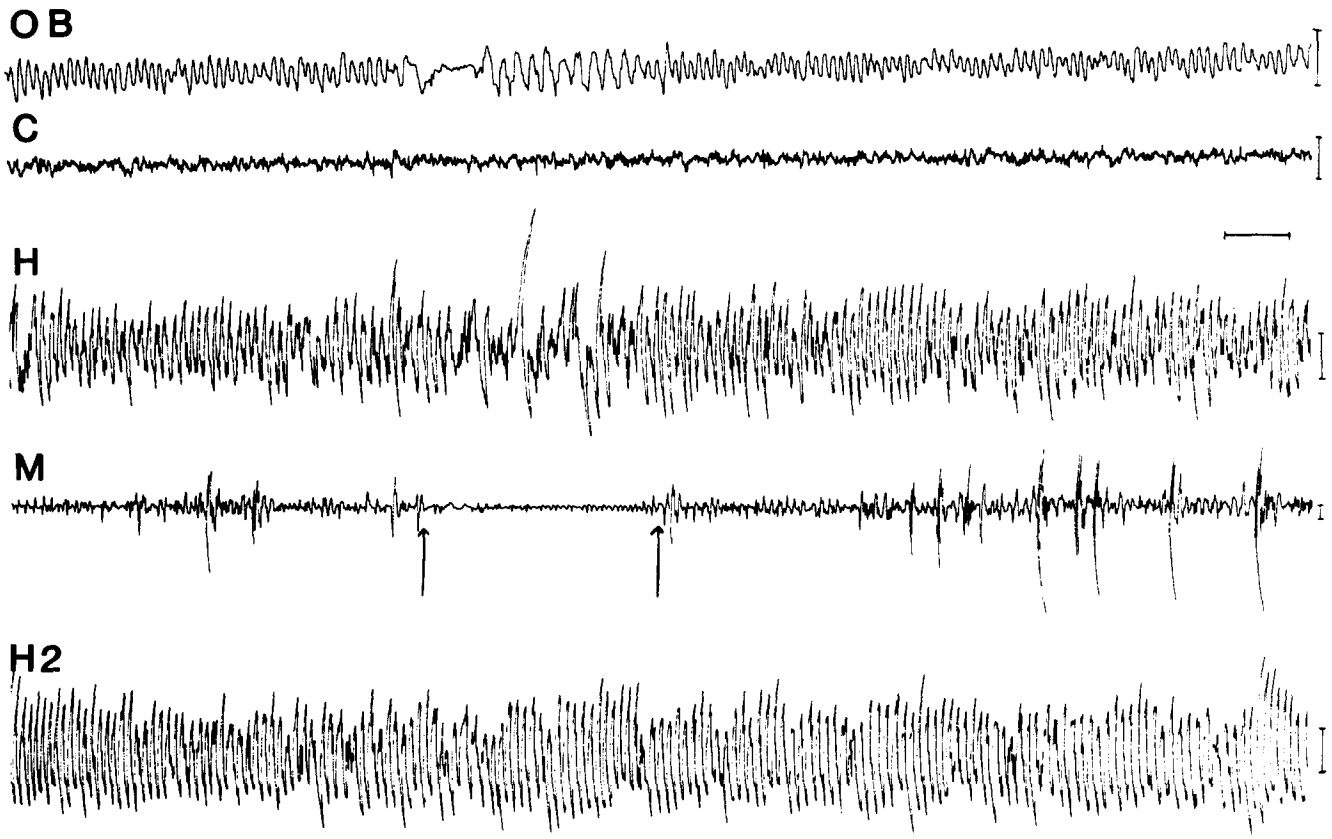

FIG. 3. Slow wave activity of various brain structures following apomorphine HCl (1.25 mg/kg, SC). OB, olfactory bulb; C, frontal neocortex; H, hippocampus; M, output from magnet and coil type of movement sensor. Top 4 traces were recorded simultaneously in rat No. 1689. Note changes in OB and H during a brief period of immobility (between arrows). H2, hippocampal activity during continuous head movement, walking and sniffing following apomorphine treatment in rat No. 1685. Cal.: 1 sec, 0.5 mV.

frequency occurred in all rats tested. Hippocampal activity was quite irregular if the rats remained motionless but RSA was present if the rats struggled while being held. Holding the rat around the upper part of the body with only the hind feet in contact with the table, or merely pressing down lightly on the rat's back as it stood on the table, also suppressed sniffing. Lifting only the hindquarters off platform (either by holding the pelvic region or the tail) had no significant effect (Table 1). These observations suggest that pressure on the anterior parts of the body actively suppresses apomorphine induced sniffing. Moreover, it is possible that contact of the vibrissae or snout with a surface is a necessary condition for the occurrence of apomorphine-induced sniffing. However, the latter possibility is less likely than the former since: (1) providing snout contact and odorous material failed to elicit sniffing when the apomorphine-treated rats were held in the hand; and (2) sniffing often persisted if the rats were lifted by the tail allowing the head and limbs to hang free. The second observation is somewhat complicated by the fact that the forepaws often (but not always) made contact with the snout when the rats were suspended by the tail.

When other observations had been completed, two of the rats were given a larger dose of apomorphine (5 or 3.75 mg/kg) in order to observe brain activity during drug-induced gnawing. The rats were placed on a heavy wire mesh screen (the floor of an overturned rat cage). The patterns of respiration and RSA were similar to those seen after the lower dose of apomorphine. However, during prolonged episodes of biting, in the absence of head movement or stepping, hippocampal activity became quite irregular and the respiratory rate was often as low as 1 Hz. The vibrissae did not move during biting.

DISCUSSION

Normal undrugged rats, while exploring a novel object, engaged in walking and head movement and in rhythmical respiratory and vibrissal movements. The respiratory movements had a mean frequency of about 7.5 Hz if the snout was in contact with the object, or if a strong odor was present but no snout contact occured. However, if sniffing occured in the absence of snout contact or strong odors, the mean respiratory frequency was only about 6 Hz. Rhythmical EMG bursts beneath the mystacial pad were often phase-locked to rhythmical respiration-related potentials in the olfactory bulb, a finding which is consistent with a previous cinematic analysis of sniffing by Welker [11].

In undrugged rats RSA occurs in the hippocampus in correlation with walking and head movement regardless of whether sniffing is present or not. A pattern of large amplitude irregular activity and sharp waves occurs during immobility, also regardless of whether sniffing is present or not [10]. If hippocampal RSA and sniffing occur concurrently, the present results show that the two rhythms may differ in frequency by as much as 2 Hz. Despite this, there is some tendency for the two rhythms to become entrained under some conditions [4].

In the neocortex, a continuous pattern of low voltage fast activity is usually present throughout a period of exploratory behavior. There is no obvious correlation between the details of behavior and the pattern of neocortical slow wave activity except that spindle activity may occur during waking immobility (often accompanied by vibrissae tremor; see also Semba *et al.* [6]) but never occurs during head movement and walking [9].

The present results indicate that apomorphine elicits a motor pattern with a strong resemblance to normal contact sniffing. The snout remained in constant contact with the substrate (as shown previously by Szechtman *et al.* [8]), the vibrissae moved rhythmically, rhythmical bursts of EMG activity could be recorded beneath the mystacial pad, and respiration consisted of an almost continuous polypnea at a mean frequency of 7.5 Hz. Hippocampal RSA was also present almost continuously, presumably in correlation with head movements and locomotion since irregular activity and sharp waves replaced RSA during brief periods of spontaneous immobility.

However apomorphine-induced sniffing differed markedly from normal sniffing in its sensitivity to environmental stimuli. As shown by Welker [11] normal rats can be induced to sniff by stimulation of any sense modality. Sniffing is generally directed toward novel objects or odors. In contrast, the apomorphine treated rats ignored objects held near them and continued their stereotyped snifffing without noticeable alteration. Following Welker [11] one might speculate that brain circuitry which coordinates the various subcomponents of the overall sniffing pattern (respiration, movements of the nose, head and vibrissae), is normally activated by varied sensory input. It appears that apomorphine can also activate these sniffing circuits, presumably by stimulation of dopaminergic post-synaptic receptors. Our results suggest, however, that this drug-induced dopaminergic input is so strong that it cannot be overridden by visual and olfactory input. On the other hand, another input, tactile stimulation of the rostral part of the body, can inhibit sniffing in the apomorphine-treated rats as it appears to do in normal rats. Sniffing behaviour also disappears during gnawing in the apomorphine-treated rat.

In conclusion, we have shown that apomorphine elicits in rats a motor pattern which resembles normal contact sniffing very closely but which, unlike sniffing, is relatively impervious to control by visual and olfactory stimuli.

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