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Operant Conditioning of Pontine Gigantocellular Units[1]

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BREEDLOVE, S. M., D. J. MCGINTY AND J. M. SIEGEL. *Operant conditioning of pontine gigantocellular units.* BRAIN RES. BULL. 4(5) 663-667, 1979.—In order to investigate the behavioral role of pontine gigantocellular field (FTG) units, we have operantly reinforced discharge in these cells in unrestrained cats using lateral hypothalamic stimulation as the reinforcing stimulus. Cats readily learned to increase discharge rate in reinforced FTG cells relative to simultaneously recorded, non-reinforced control cells. Increased FTG discharge was associated with elevated levels of motor activity and often with stereotyped movements. Our results are compatible with the hypothesis that FTG unit discharge is related to specific movements.

Reticular formation Motor activity

A WIDE range of functions has been ascribed to the cells in the gigantocellular tegmental field (FTG) [2] of the pontine reticular formation, including control of REM sleep [5], eye movements [4], mediation of arousal [1], and other complex behaviors [7]. Many of these conclusions were based on studies of restrained or anesthetized animal preparations. Previous studies in this laboratory [8,9], using awake, unrestrained cats, have suggested that changes in FTG neuronal unit discharge are associated with specific movements.

Operant conditioning of augmented unit discharge provides an objective method for documenting behavioral relations. Increases in cell discharge should be accompanied by an increase in behaviors which are closely correlated with cellular activity. The purpose of the present study was to determine whether FTG discharge could be increased using operant techniques, and to investigate the types of behavioral changes accompanying such increased discharge.

METHOD

Three adult female cats were anesthetized with 35 mg/kg, IP sodium pentobarbital (Nembutal). A pair of stainless steel cortical screws were implanted in the skull over the sigmoid gyrus for electroencephalographic (EEG) monitoring and behind the orbit for electrooculographic (EOG) monitoring. A pair of stranded stainless steel wires was implanted approximately 2 cm into dorsal neck muscles for recording electromyographic (EMG) activity. Tripolar macrolectrodes were implanted bilaterally in the lateral geniculate nucleus (LGN) at stereotaxic coordinates A 6.0, L 10.0, and H 2.0-3.0 and lateral hypothalamus (LH) at coordinates A 9.0, L 3.0, and H —3.8. Electrodes were connected to a 20-pin Winchester plug which was affixed to the skull with dental cement.

Single units were recorded from 32 μ stainless steel microwires mounted on previously described mechanical microdrives [3] which propelled the microwires through the FTG. The microdrives were oriented 40° from the vertical in the saggital plane to avoid the bony tentorium. Each cat was implanted with two microdrives, each of which propelled two bundles of seven microwires.

Recordings were made in a shielded, sound attenuated 58x60x85 cm chamber while the cats were completely unrestrained and freely moving. A glass door mounted on the front of the chamber allowed observation of the animal. Only units with stable spike amplitudes and signal-to-noise ratios of 3:1 or better were recorded. Cell activity was monitored with a Tektronics 565 oscilloscope and a window discriminator. The window discriminator pulse output was recorded on a Grass model 6 polygraph along with EMG, EOG and EEG signals. The window discriminator output also drove an audio monitor and was recorded on an electronic counter with printout every 10 sec, or a cumulative recorder which reset every 10 sec. Polygraphic measures and amplified unit signals were also recorded on magnetic tape. Each cell was observed during at least one sleep cycle, i.e., waking (W), slow wave sleep (SWS) and rapid eye movement sleep (REM). On the basis of the firing pattern during the sleep cycle, each cell was classified as type 1, 2, or 3 in the system described previously [11]. Type 1 cells had no background activity, firing in association with spontaneous or stimulus-evoked movement. Type 2 cells had high (>4 spikes/sec) tonic activity in W, SWS, and REM which increased during movement. Type 3 cells had a low level of

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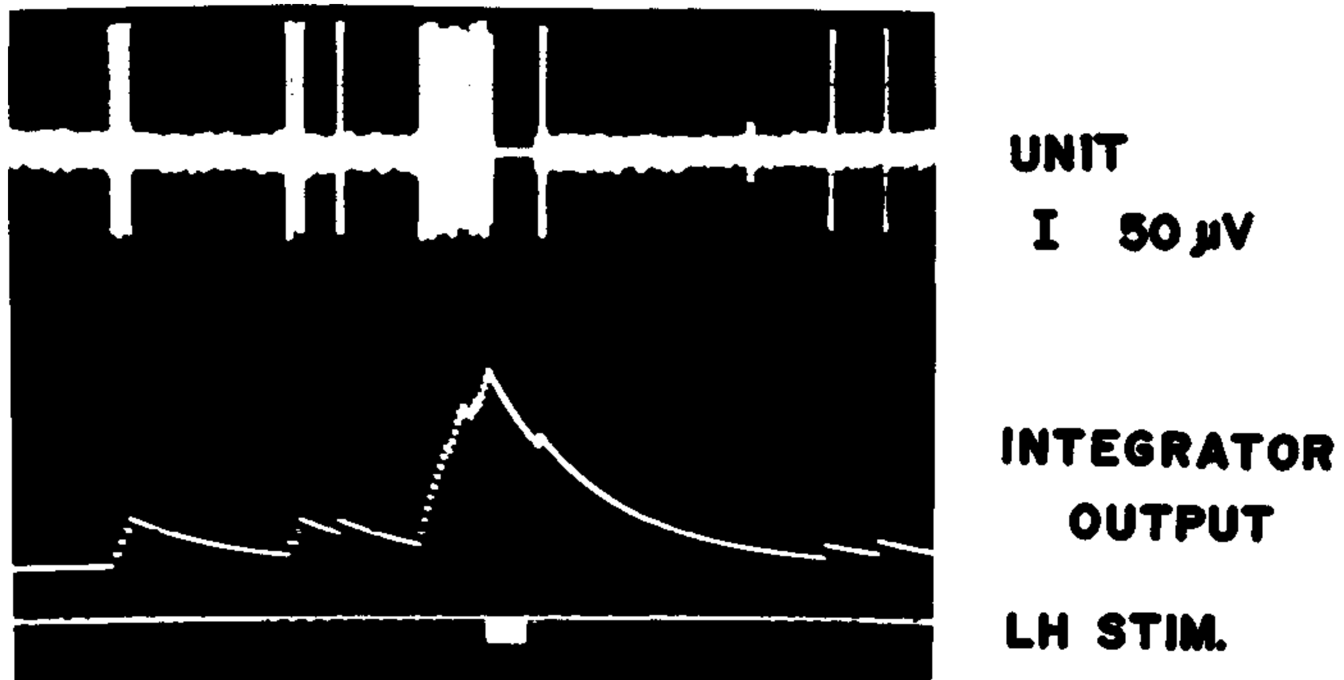

FIG. 1. Method of operant conditioning of FTG cells. Upper trace: amplified unit output. Middle trace: integration of unit activity. Bottom trace: LH stimulation record (400 msec).

spontaneous discharge during quiet waking and SWS (<4 spikes/sec), but fired in phasic bursts during active waking and REM. The waking behavioral correlate of discharge was determined for each cell by systematically presenting stimuli and observing spontaneous motor behavior [8].

Increased unit discharge was operantly reinforced by LH stimulation, consisting of a 400 msec train of pulses 0.3 msec in duration delivered at a frequency of 100 Hz, and an amplitude of 4.0 to 6.0 V. In each cat the reinforcing value of the LH stimulation was initially confirmed by conditioning the prolonged absence of eye movements as determined by monitoring of the polygraphic EOG recording. The cats readily learned to perform this task by sitting quietly with eyes fixated for up to 60 sec to receive LH stimulation.

During FTG unit reinforcement, the window discriminator output was electronically integrated and LH stimulation delivered when the integrator output reached a predetermined threshold. Figure 1 illustrates the method of reinforcement. Each unit spike on the upper trace increased the integrator output (middle trace) until it reached the predetermined threshold value, triggering the LH stimulation indicated on the bottom trace. During the LH stimulation, a relay grounded the unit recording amplifier output to prevent artifactual spike counts. Note that the integrator output decreases in the absence of unit activity. Unit firing was shaped by slowly raising the threshold necessary for LH stimulation, thereby increasing the rate of firing required for reinforcement. One of two conditioning paradigms was used. During initial experiments a long (40 min) reinforcement session with a 10 min baseline (no reinforcement) period before and after was used. However, it was found that performance decreased at the end of long sessions and therefore later experiments consisted of four 10 min reinforcement sessions alternating with five 10 min baseline periods. The cats were presented with a 60 W light bulb visible through the glass door of the chamber, as a discriminative stimulus signalling reinforcement periods. During the reinforcement of twelve experimental cells, a second, control cell was recorded simultaneously.

After the microdrives were advanced the planned distance through the FTG, the cats were sacrificed with an overdose of Nembutal and a 15 sec, 15 µA current passed through each microwire used for recording. Iron deposited by this current was later stained by Gomori's reaction. The brain was intracardially perfused with 10% Formalin, sectioned in 80 µ slices in the saggital plane and Nissl stained. Anatomical placement of each cell was determined.

RESULTS

Twenty-two cells histologically verified to be within the FTG were reinforced. Stereotaxic coordinates of these cells were from P 2.5 to P 5.5, and L 1.2 to L 2.3 within the FTG as labeled by Berman [2]. FTG discharge was significantly higher during the reinforcement sessions ($p<0.01$ one-way analysis of variance, $N=22$). Sixteen of the twenty-two cells reinforced had significantly higher mean 10 sec rates during reinforcement sessions than they did in baseline sessions ($p<0.05$, two-tailed, *t*-test). Of the remaining six cells, two displayed significantly less firing during reinforcement sessions, and four showed no significant change in discharge rate during reinforcement. Performance within a reinforcement session was somewhat variable, as is illustrated in Fig. 2. Note the increased discharge during reinforcement sessions and the extinction curves during the interposed baseline sessions.

Of the twenty-two cells, 3 were type 1, 9 were type 2, and 10 were type 3. The mean discharge during baseline sessions was 2.0 spikes/sec for type 1 cells, 13.9/sec for type 2 cells, and 5.6/sec for type 3 cells. The mean discharge rate during

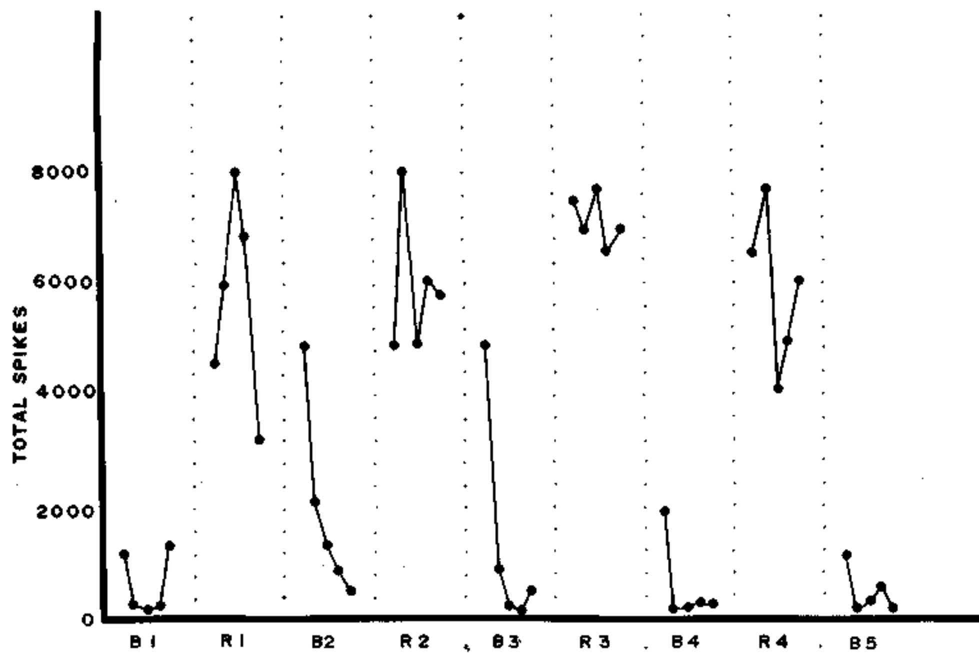

FIG. 2. Unit discharge of a reinforced FTG cell. Each point represents 2 minutes. B=baseline (no reinforcement) periods; R=reinforcement periods. The cat was presented with a discriminative stimulus signaling reinforcement periods. Note increased discharge during reinforcement and successive extinction curves in baseline periods.

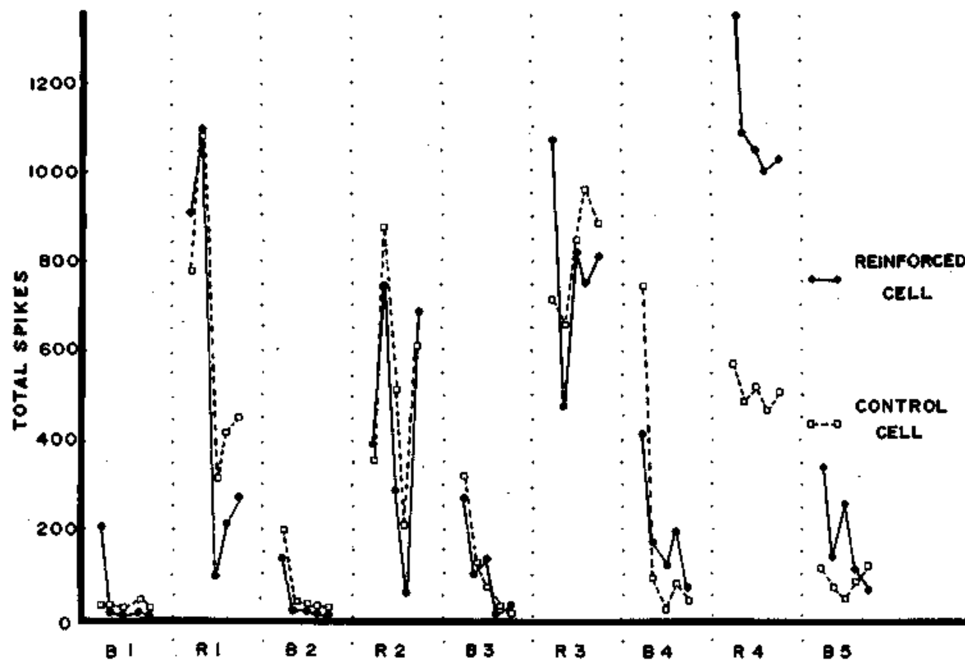

FIG. 3. Unit discharge of a reinforced FTG cell and a control FTG cell. Each point=2 min. B=baseline periods. R=reinforcement periods. Note higher discharge of reinforced cell in the final 10 min of reinforcement (R4).

TABLE 1

PERCENTAGE INCREASE IN MEAN 10 SEC DISCHARGE FROM BASELINE TO THE FINAL TEN MIN OF REINFORCEMENT, COMPARING THE EXPERIMENTAL (REINFORCED) CELL WITH A CONTROL (UNREINFORCED) CELL RECORDED SIMULTANEOUSLY. THE EXPERIMENTAL CELLS HAD SIGNIFICANTLY HIGHER PERCENTAGE RATE INCREASES THAN THEIR MATCHED CONTROL CELLS (WILCOXONSIGNED-RANK, MATCHED-PAIRS TEST,p<0.05, TWO-TAILED).

Experimental Cells	Control Cells
+218.13	+ 94.69
+313.26	- 93.68
+ 170.28	+ 151.94
+ 114.74	+302.07
+ 26.99	+ 120.78
- 15.41	- 38.43
+276.26	- 40.73
+ 30.48	- 32.37
- 72.37	- 93.37
+608.92	+303.14
+356.93	+ 92.48
+514.67	+ 30.02

reinforcement sessions was 6.0 spikes/sec for type 1 cells, 25.8/sec for type 2 cells and 15.9/sec for type 3 cells. The mean maximum 10 sec discharge rate of type 2 and 3 cells during reinforcement was 44.52 spikes/sec. This is higher than the previously reported mean REM rate of 17.36 spikes/sec in these cells [11]. (Type 1 cells, by definition, do not discharge in REM sleep.)

During the reinforcement of twelve experimental cells, a second, control cell was recorded simultaneously. The mean 10 sec firing rate during baseline periods was not significantly different in experimental and control cells (matched pair?-testp>0.20). Of the eleven pairs of cells recorded (one pair was used a second time, with the roles of experimental and control cells reversed), the pre-reinforcement discharge was significantly correlated in six of the pairs (*t*-test, p<0.05). All significant correlations were positive. Comparison of unit discharge change in experimental and control cells demonstrated the specificity of the conditioning procedure. Figure 3 shows an experiment of this type. Note that both the experimental cells and the control cells increased discharge during the early reinforcement periods. This period was often accompanied by active exploratory behavior. But during the final 10 min of reinforcement, the experimental cells maintained or increased discharge while the discharge of control cells was reduced to near baseline levels. At this point cats often made relatively discrete movements, rather than showing the non-specific activity increase seen earlier in training. If the discharge of the entire reinforcement session was averaged, the percentage of increase over baseline was not significantly different in experimental and control cells. However, the percentage of increase in discharge during the final 10 min of the reinforcement session was significantly greater in experimental cells than in control cells (p<0.05, two-tailed, Wilcoxon sign-rank, matched-pairs test). Table 1 compares the experimental and control cells in terms of percentage of increase in discharge during the final 10 min of reinforcement.

For all 22 FTG cells reinforced, increased discharge was accompanied by an increase in general motor behavior. For twelve of these cells, the cat displayed a more specific movement pattern, typically involving head or neck movement, during the final part of the reinforcement session. For example, by the end of the conditioning of one cell the cat was sitting quietly bobbing its head up and down. Before conditioning, this cell was observed to fire whenever the animal moved its head up. During the conditioning of another cell, the cat continuously turned its head and body to the left, traveling in a circle within the chamber. Before conditioning, this cell was observed to fire when the animal turned its head to the left. Thus movement associated with conditioning corresponded well with the previously determined behavioral correlate of discharge.

DISCUSSION

FTG unit discharge could be increased in unrestrained cats, using operant conditioning techniques. That the increased pontine FTG firing was not due simply to general FTG activation from the LH stimulation, as has been reported in medullary reticular formation units [6], is indicated by the fact that during the conditioning of twelve cells, another, control cell showed a significantly smaller percentage of increase in discharge during the final portion of reinforcement. In six cases, the control cell and experimental cells were recorded from different wires of the same bundle and therefore were within approximately 200 μ of each other. Furthermore, in six of the eleven pairs, the control and experimental cell discharge was positively correlated during baseline periods. Particularly in these cases, the differential change in discharge rate of the two cells in response to LH stimulation, despite their anatomical proximity, and their normally positively correlated discharge, argues against a general effect of hypothalamic stimulation. Finally, because of the operant contingency imposed, increased FTG discharge did not follow, but preceded each LH stimulation. This is illustrated in Fig. 1.

That repeated LH stimulation need not necessarily be accompanied by increased movement was demonstrated in each cat during the initial step of reinforcement of absence of eye movements. The animals learned to perform this task by sitting quietly with eyes fixated. Since FTG discharge is related to movement [9,10], it is not surprising that the cat at first learned to gain reinforcement by general movement, thereby increasing the firing rate of many FTG cells, including both the experimental and control cells. This increase in activity was responsible for the positive correlation of simultaneously recorded FTG cells during baseline periods. As the conditioning continued, the animal learned to increase discharge more selectively in the experimental unit. Therefore the control and the experimental cells increased an equal amount in the early portion of the reinforcement period, while the experimental cells discharged significantly more than the control cells in the later part of the reinforcement.

FTG cells have been called the best candidates for the executive neurons for REM sleep because these cells fire more during REM sleep than at any other time in a head-restrained preparation [5]. However, an operantly conditioned increase in FTG firing was not accompanied by REM sleep or cataplexy. Similarly, we saw no selective change in eye movements, or complex behaviors that have

been associated with activity in reticular formation units. Because cats readily learned to increase FTG discharge rate, it is unlikely that unit discharge was related to pain or aver-sive responses.

In conclusion, we found that cats can be operantly conditioned to increase FTG discharge. FTG firing was accompanied by increased movement, often of a specific nature unique to the cell, and in agreement with the behavioral correlate of discharge seen outside the conditoning sessions. These results are compatible with the hypothesis that discharge in individual FTG cells is related to specific movements.

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