Effect of nucleus accumbens destruction in rat[1]

G. Fantin and D. Bottecchia

Istituto di Fisiologia Umana dell'Università di Padova, Via Marzolo 3, 35100 Padova (Italy), 17 March 1983

Summary. The paper presents the effects of nucleus accumbens destruction in rats. There are certain behavioral correlates (e.g., avoidance learning and dominance) which are influenced by the destruction of the nucleus accumbens, while other specific correlates are not significantly affected (namely, open field movements and competition for food). The results are discussed.

There is evidence that an important function of the motor system is to organize and coordinate the activities of indivdual muscles so as to generate sequences of movements[2]. According to Graybiel[3], the nucleus accumbens is a key structure in functionally linking motivation and action to the interface of the limbic system with motor mechanism(s). It has been reported that dopamine injection into this nucleus can initiate locomotor responses in rats[4]; however, the injection of dopamine antagonists has been reported to increase the aforementioned responses[5].

There are neural connections from the nucleus accumbens to the globus pallidus[6]. These fibers seem to be GABA-ergic[7], and they appear to be involved in the initiation of locomotive responses. The purpose of the present study was to investigate the physiological significance of the nucleus accumbens, and our results indicate that the output from this structure modifies certain specific behavioral correlates in the rat.

Materials and methods. Albino Wistar rats were used (body weights from 190 to 320 g); each experimental trial was initiated 7 days after elective destruction of the nucleus accumbens.

Lesion procedure. The rats were anesthetized with urethane nembutal[8] and then fixed to a stereotaxic instrument. After the scalp was opened and the skull was bored (by using a dentist's drill), a thin electrode (0.2 mm diameter) was inserted until the tip arrived at the level of the nucleus accumbens, according to the coordinate system of de Groot[9,10]. The electrodes were coated with Cramolin Plastik P. (R. Schafer and Co., Muhlacker, Germany), an electric insulator which is effective up to 25 kV; care was taken to avoid coating the last 0.5 mm near the point of the electrode. Once the electrode was positioned, a second one was connected to the tail of the animal. The 2 electrodes were then connected to a lesion maker (LM 3 – Grass Instrument Co., Quincy, Mass., USA) and the electrical current was allowed to pass through the circuit. Nucleus accumbens destruction was performed bilaterally. In a preliminary group of experiments, we selected the intensity and duration of the current which would produce optimal effects (namely, lesions limited to the nucleus accumbens). At the conclusion of each experimental trial the rats were sacrificed with an overdose of ether. The brain was removed and quickly frozen by immersing it into liquid nitrogen, and then sectioned at 20 µm with a cryostat. Every fifth section was saved and then stained with hematoxylin and eosin for later histological analysis.

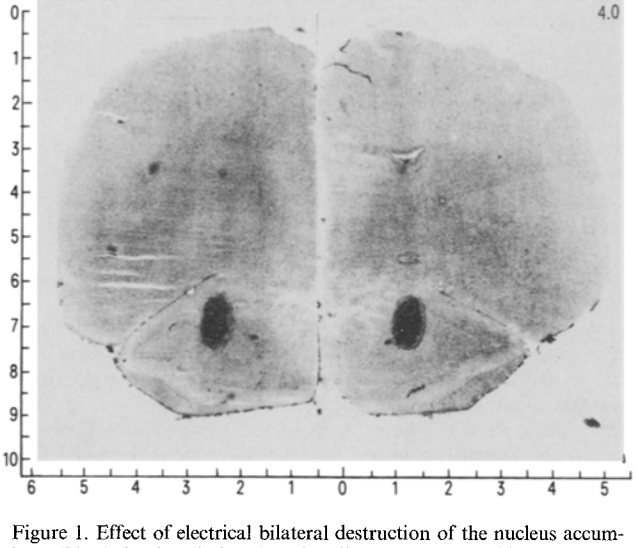

Figure 1. Effect of electrical bilateral destruction of the nucleus accumbens. The lesion was induced as described in the text. This animal was sacrificed 1 h after treatment, and was not used for experiments. The upper right hand number (4.0) corresponds to the number of mm anterior to the reference point (skull landmark bregma); the ordinate indicates the distance in mm from the dura; the abscissa gives the distance in mm from the midline.

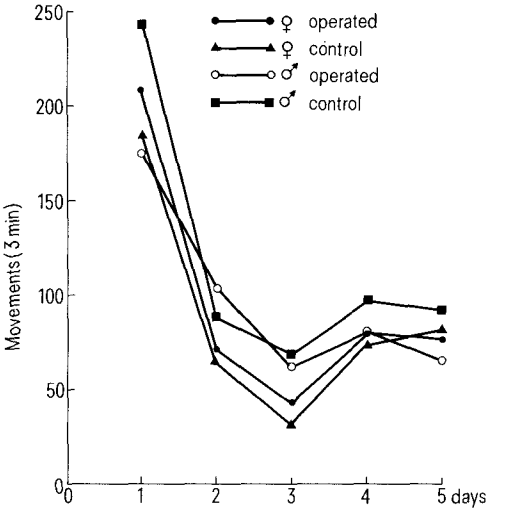

Figure 2. Movements recorded in an open field for 3 min daily over 5 consecutive days (in 2 equally divided groups of males and females consisting of 5 normal controls and 5 denucleated specimens each).

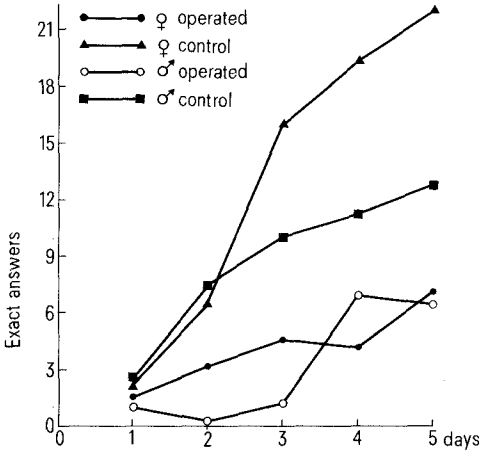

Figure 3. Avoidance learning test: number of correct responses recorded daily for 5 consecutive days in normal controls and in denucleated animals (5 males and 5 females).

Locomotor behavior in open field. Each animal was tested in an open field for 3 min every day for 5 consecutive days. The apparatus (Activity Cage Control A3 – Basile, Milano, Italy) measured 36 × 23 × 20 cm, and the floor consisted of metal bars (spaced 1 cm apart) so that each time the rat shifted a foot from one bar to another an electric signal was registered.

Avoidance learning. The rats were first habituated to the shuttle box avoidance apparatus for 10 min of free exploration. This apparatus consisted of 2 compartments measuring 24 × 21 × 21 cm each, separated by a wall with an opening of 7 × 8 cm. Location sensor microswitches, affixed under the grids, recorded crossing movements. The grids produced a scrambled shock of 1.0 mA at 350 V by means of a shocker connected to the avoidance apparatus. On a random schedule (X = 20 sec), the conditioned stimulus (an electric lamp) was activated for 5 sec, followed by a foot shock which was maintained until the animal entered the other compartment. If the rat failed to move, the shock was automatically terminated after 10 sec. On the other hand, if the rat crossed over the other side during presentation of the conditioned stimulus, the light switched off and no shock occurred. Only this latter case was recorded as a correct response. A chronometer monitored the time interval between the presentation of the conditioned stimulus and entry into the other chamber (or the cessation of the electric shock). This period was defined as the waiting time. Each rat received 30 tone-shock trials a day for 5 consecutive days.

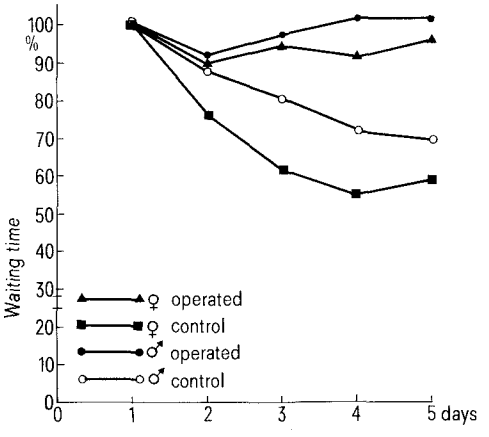

Figure 4. Total waiting time recorded daily over 5 consecutive days in normal controls and in denucleated animals (5 males and 5 females in each group). The waiting time is expressed as % of this time recorded during the first day.

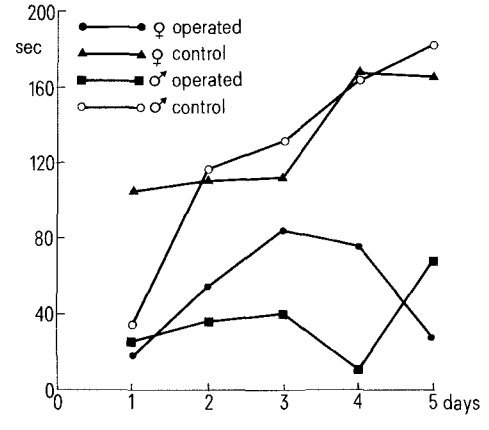

Figure 5. Dominance test: time spent on the platform by the normal controls and denucleated animals (5 males and 5 females in each group) on each day for 5 consecutive days during the dominance test (3 min).

Dominance test. The apparatus used to test dominance consisted of a 48 × 21 × 21 cm plastic cage fitted with a flooring of metal grids which were used to deliver a foot shock. A transparent plexiglass cover, and a mirror attached to a wall next to the enclosure, permitted the animals to be observed inside the cage. A platform was fitted to one wall of the compartment, 10 cm above the floor. In each experimental task a 5 MA current was passed through the grid (for 3 min) as an aversive stimulus, and the time spent by each rat on the platform was measured with a chronometer. The small size of this raised structure allowed only 1 rat to stand on it. Each animal received a period of initial training in the chamber before the actual experiment began. Criterion was reached when an animal demonstrated a successful avoidance in less than 45 sec in 2 consecutive trials; this initial training was carried out in order to ensure that each animal had learned an adequate avoidance behavior. A round-robin procedure was instituted in order to permit dyadic comparisons to be made. Each pair of rats was tested only once in the dominance chamber, and each subject was tested once a day.

Competition for food. 2 animals, 1 denucleated and 1 normal control, were placed in a box containing a small metal tray in one corner which served as a food dish. The size of this receptacle was such as to permit only one animal at a time to have access to the food. Liquid alimentation was employed in order to prevent the animals from removing the food from the dish; the rats were habituated to this type of nourishment prior to the test trial, and all subjects were deprived of food for 72 h prior to experimentation. Dominance was measured by the time that each rat spent eating at the dish. The animals were tested for 15 min on each of 2 successive days, and each subject was paired with all the other members of the group.

Results. Figure 1 shows the effects produced by the bilateral electrical destruction of the nucleus accumbens. As one can see, the lesion is limited to the nucleus, which has been replaced by necrotic material and fibrin fibers.

Males and females were divided into 2 equal groups, each consisting of 5 controls and 5 nucleus accumbens-operated representatives. Figure 2 shows that there were no open field behavioral differences between the controls and the operated animals. Acquisition results for the avoidance learning study are given in figure 3. The control group had a faster rate of acquisition. This result became increasingly evident as time passed; on the fifth day the operated males showed 6.4 ± 3.8 correct responses, while the controls showed 12.8 ± 4.7 ($p < 0.05$). The operated females had 7.2 ± 3.6 correct responses and the controls 22 ± 4.1 ($p < 0.001$). Similarly, the total waiting time decreased for the controls but not for the nucleus accumbens-operated animals: figure 4 shows that on the fifth day the 5 operated males had a waiting time corresponding to 102.3% ± 19.8 with respect to that of the first day, while the 5 control males showed 69.4% ± 6.9. Analogously, the 5 operated females had a waiting time of 96.3% ± 12.7 on the fifth day and the 5 female controls were registered as 59.7% ± 7.3. Figure 5 shows the results of the dominance test: the nucleus accumbens-operated rats spent significantly less time on the platform than did the normal control group. On the fifth day the operated males spent 67.8 ± 18.1 sec on the platform and the controls 183.8 ± 37.6 sec ($p < 0.05$); similarly, the operated females spent 27.8 ± 12.3 sec and the controls 167.4 ± 35.6 sec ($p < 0.05$). In fine, there were no significant differences between the controls and the nucleus accumbens-operated rats under conditions of food competition.

Discussion. Our results indicate that there are certain behavioral correlates (e.g., avoidance learning and dominance) which are influenced by the destruction of the nucleus accumbens, while other specivic correlates are not significantly effected (namely, open field movements and competition for food). While the motivation for action is influenced by a very aversive situation, such as a foot shock, the animal does re-

quire a complex neurologic integration involving the nucleus accumbens in order to perform intentional movements, whereas this kind of integration is not necessary when the motivation for movement is not linked to some aversive situation.

Little is known about the exact mechanisms by which the limbic processes gain access to the motor system. The nucleus accumbens receives direct connections from the amygdala, hippocampus and other limbic forebrain structures, as well as indirect connections via the mesolimbic dopaminergic projections from the ventral tegmental area of Tsai. Furthermore, it also has direct and indirect connections to the globus pallidus via the substantia nigra and the nigrostriatal dopaminergic system[2, 11-18]. Therefore, this nucleus appears, on anatomical grounds, to be part of a functional link between the limbic system and the basal ganglia[3]. The fact that the nucleus accumbens has anatomical connections with both the motor and limbic systems, plus the experimental evidence indicating that destruction of this nucleus modifies some complex motor responses to aversive stimuli involving both the emotional status and the motor system of the animal, are clear indications of the importance of this nucleus in the control of locomotor responses. However, motor activity in resting conditions (open field movements) is not modified by destruction of this nucleus, thus indicating that not all motor activities are regulated by this pathway.

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Immunoactive TSH in the amniotic fluid of the rat[1]

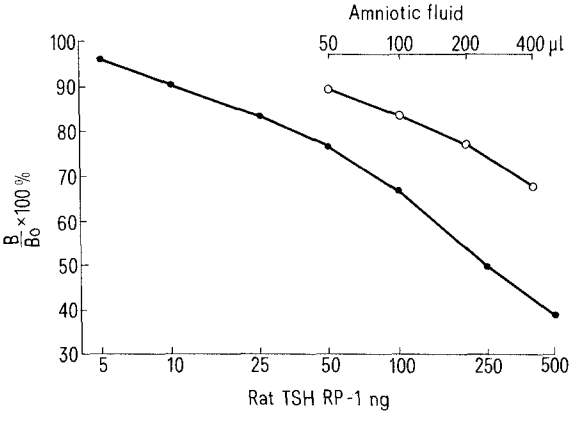
F. Tang and P.Y.D. Wong[2]

Department of Physiology, Faculty of Medicine, University of Hong Kong (Hong Kong), 16 May 1983

Summary. Amniotic fluid was obtained from 19-day-old rat fetuses by aspiration. Pooled samples measured at 4 different dilutions demonstrated parallelism with standard rat TSH. It is concluded that rat amniotic fluid has TSH immunoactivity.

Pituitary hormones, like prolactin[3] and corticotrophin[4], have been reported in human amniotic fluid. More recently, thyrotrophin has been found in the amniotic fluid of the lamb[5] and human fetuses[6]. Thyrotrophin as well as thyroid hormones have also been measured in the blood of 21-day-old rat fetuses[7]. However, these hormones were undetectable in the amniotic fluid of rat fetuses at term[8]. In this study, we report the estimations of TSH immunoreactivity in the amniotic fluid of 19-day-old rat fetuses.

Materials and methods. Young virgin adult female Sprague-Dawley rats weighing about 200 g were allowed to mate with young adult males. Vaginal smears were made each morning and the day that spermatozoa were found in the smears was taken as day 0 of pregnancy. Altogether 7 pregnant rats were used in 2 separate experiments. On day 19 of pregnancy, the mothers were killed with ether and the fetuses were quickly dissected out and put on ice. Amniotic fluid was obtained by aspiration. Due care was taken to avoid contamination of the sample with blood. Amniotic fluid from 5–10 fetuses was pooled and was stored at −20 °C prior to radio-immunoassay. Aliquots of 0.2 ml were measured in duplicate using a rat TSH RIA kit kindly supplied by the National Institute of Arthritis, Metabolism, and Digestive Diseases. The sensitivity of the assay was about 10 ng, and the intra-assay and inter-assay coefficients of variation were about 3% and 10% respectively. The antibody was highly specific for rat TSH and did not cross-react significantly with any other rat pituitary hormones. In order to establish parallelism between amniotic fluid and



Dilutions of pooled amniotic fluid showed parallelism with standard rat TSH.