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The Effects of d-Amphetamine on the Electrophysiological Activity of the Superior Colliculus in the Rat

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CHEE, S.-J. *The effects of d-amphetamine on the electrophysiological activity of the superior colliculus in the rat.* PHARMACOL BIOCHEM BEHAV **40**(2) 215–220, 1991.—The superior colliculus (SC) is thought to have an inhibitory effect on arousal (and hence activity) in rats when light is present. d-Amphetamine is believed to suppress this inhibition of the SC on arousal. The present study investigated the electrophysiological activity of the SC of the rat under the influence of d-amphetamine in both light and dark conditions. A single numerical arousal index value (AI) for the electrophysiological data obtained by dividing the frequency of the waves by the voltage over a specified interval was used. Results showed that light conditions decreased the AI of the SC. The AI was, however, not affected by d-amphetamine. Also, the electrophysiological activity of the SC showed spontaneous emissions of high-voltage (600 μV) alpha waves which generally occurred in two- to four-second bursts. Occurrence of this high-voltage activity was reduced by increasing doses of d-amphetamine.

EEG Superior colliculus d-Amphetamine Illumination Locomotor activity Arousal Hooded rats

AMPHETAMINE has been labelled as having properties that promote an increase in locomotor activity in several mammalian species, including humans, by activating processes of the central nervous system (CNS). Knowledge is insufficient, however, about how this drug alters the state of CNS functioning.

Alexander and Isaac (1) found that when d-amphetamine (dA) was given orally to rhesus monkeys (a diurnal animal), a decrease in locomotor activity was observed as the dose level of the drug was increased. The investigators attributed this contradictory effect of amphetamine to the fact that the drug exerted its influence by reducing the influence of light on the nervous system, thereby lowering the arousal level and producing a decrease in activity. Conversely, owl monkeys (13) and rats (29), which are nocturnal animals, exhibited an increase in activity in light conditions when given dA.

The change in level of locomotor activity in animals was thought to be a result of sensory input into the brainstem reticular formation system (BSRF) (9, 11, 12). Since amphetamine is thought to exert its effects via the BSRF (31), it could be postulated that the drug's depressant effect on the diurnal animal is a result of an attenuation of sensory input into the BSRF which results in a lowering of the arousal level leading to a decrease in locomotor activity and vice versa for nocturnal animals. Researchers have also found a relationship between sensory stimulation via the visual system and dA (24, 29, 30). Goestch and Isaac (8) posited that dA may have a direct effect on the visual system, acting to suppress visual input. Isaac (10) found that lesions of the superior colliculi (SC) produced a large increase in activity and proposed that " . . . input to the dorsal tegmentum from the superior colliculus is inhibitory in nature" (p. 159), and concluded that the overall increase in activity that was seen with collicular lesions was due to a release of this inhibitory mechanism of the SC. In the same study, Isaac gave the rats increasing doses of dA and found that the increase in activity in rats with collicular lesions correlated directly with increases of drug dosages. This was further evidence that the SC had an inhibitory effect on arousal when exposed to light and that dA acted to reduce this collicular inhibition.

The SC has rich fiber connections with the BSRF (3). There are, unfortunately, few data that explicitly document the relationship between the SC and the reticular structures in terms of arousal levels as measured physiologically or by means of overt behavioral responses.

Altman (2) revealed that lesions of the SC can disrupt the balance of day-night activity cycle in rats. Kallman and Isaac (14) suggested that the SC activity is light dependent and involved in altering locomotor activity.

Moruzzi and Magoun (19) noted that during sleep, EEG patterns were seen to consist of high-voltage slow waves. The pattern changed to low-voltage fast activity during wakefulness. Lorig and Isaac (17) suggested a numerical arousal index value for the electrophysiological data by dividing the voltage measure by the number of zero-crossings (frequency) over a specified interval. It is assumed that the electrophysiological activity in the SC functions similarly to BSRF and cortical EEG. Hence, the arousal index was used to represent collicular activity in the present study. The arousal index was modified by dividing the frequency counts of the EEG waves by the voltage. Thus a high

index value would represent a more aroused EEG pattern (high-frequency/low-voltage waves) than a low index value.

The present study investigated the electrophysiological activity of the SC of the rat under the influence of dA in both light and dark conditions. It was hypothesized that under light conditions, the drug would decrease collicular activity indicated by a lower AI. This decrease would, in addition, be directly correlated with the drug dosage and locomotor activity. Since the three dosage levels of 0.2, 0.4, and 0.8 mg/kg of dA have consistently produced behavioral changes in monkeys as well as rats and because they encompass human clinical doses (1, 29, 30), the present study continued to use these dosages.

METHOD

Subjects

The subjects were eight Long-Evans derived male hooded rats from the breeding colony of the Animal Behavioral Laboratory in the Psychology Department at the University of Georgia. All animals were from 100 to 120 days of age at the time of surgery. Each rat was housed individually and maintained on a 12-h light/12-h dark schedule with light onset at 9:00 a.m. local time. Food and water were available ad lib throughout the experiment.

EEG Signal Recording Apparatus

The testing apparatus consisted of a recording chamber (constructed from a glass aquarium) measuring $30 \times 59 \times 28$ cm located in a Faraday room. The floor of the chamber was covered with Sanicel bedding material. Incandescent lights provided 1.35 log ft lamb when on and there was less than 0.02 log ft lamb at the bottom of the recording chamber when the incandescent lights were off.

A stainless steel depth electrode (0.25 mm diameter orthodontic wire) insulated with epoxylite electrode insulator except at the tip, was soldered to a Winchester socket (type SM3S). The electrophysiological signal was carried over a two-conductor ribbon cable to a Grass EEG machine (Model 6). The recording plug was connected to the cable by a telephone cord and suspended above the subject in the center of the chamber. This allowed unrestricted movement while maintaining a slight, but constant tension on the cable. The sensitivity scale of the Grass EEG AC amplifier (Model 6A5D) was set at 15 μV/mm, and the paper speed at 15 mm/s.

EEG Data Acquisition Apparatus

The signal was taken from the J9A output to a screw board terminal (Metrabyte™ STA-8PGA) which allowed direct analog input signals via screw terminals to Input/Output lines of a data acquisition and control interface board (Metrabyte™ DAS-8PGA) which employed a 12-bit successive approximation analog-to-digital (A/D) converter with conversion times of 25 μsec. Connections to a microcomputer were made using a cable with a standard 37-pin D male connector (Metrabyte™ C-100 STA-8). All response measures were recorded and stored using Labtech Notebook™.

The signals were sampled at 70 Hz. The signal input range was set between −1 V and +1 V. Thus, for each 30-min recording session, 240 s, or 16,800 digitized data points, of EEG data were collected and stored (sampling rate of 1 Hz produced 1 digitized data point).

A Fourier smoothing of the input signal was performed using ChemSoft™ to eliminate any noise that might interfere with the original EEG signal. A computer program was written in QuickBasic™ (QB45) to analyze the amplitude (voltage) and frequency of the signal. The program identified data points that formed the maxima and minima within the wave form. The corresponding voltage values of these data points and the absolute values of the voltages were taken, and the mean of these values was calculated for 5-min intervals. The number of data points that formed the maxima were noted over 5-min intervals and the mean frequency was calculated. The AI for each interval was, thus, given by the quotient of the mean frequency and the mean voltage.

Locomotor Activity Recording Apparatus

A light beam, filtered by an infrared filter, bisected the recording chamber 2 cm above the floor and fell on a photocell, protected by an infrared filter to maintain constant sensitivity, on the opposite side of the chamber. When the beam was obstructed by the rat, a count was registered and simultaneously recorded by the polygraph (via the J9 input) as discrete pen deflections, and the microcomputer.

Surgical Procedure

Prior to surgery, each animal was habituated to handling and to the activity chamber by being placed there for 40 min (the duration of each testing session) per day for five days. Surgery was performed under sodium pentobarbital (Nembutal; 50 mg/ml) anaesthesia which was administered in the amount of 1 ml/kg body weight with an initial intraperitoneal injection followed by a total of 1.5 mg/ml atropine sulphate for each subject.

Stereotaxic coordinates were taken with bregma as the landmark reference (21). One cortical electrode, placed approximately 3 mm anterior to the left coronal suture and 2 mm lateral to the sagittal suture, was the reference electrode. The depth electrode was located over the right SC by initially placing it at AP −6.8 mm, L −1.0 mm, and H −3.5 mm. Holes in the skull were drilled using a 0.87-mm twist drill bit attached to a pin vise. To ensure that the electrode was properly localized in the SC, a strobed light at 16 Hz was presented to the left eye and the electrode was lowered at 0.1-mm increments until a visually evoked potential (Grass EEG model 6 output) was observed. Final horizontal coordinates ranged from 3.4 to 3.7 mm. The electrodes and the Winchester socket were held in place with dental acrylic.

Testing Procedure

Following surgery, handling and habituation to the test equipment continued for four days. Data collection began on the fifth day after surgery and was continued for 13 days. The subjects were randomly assigned to two sets of four rats each. The first set began with light conditions followed by alternating dark-light conditions (LD sequence). The second set of subjects started the experiment under dark conditions on the first day followed by light-dark alternations (DL sequence). This was done to determine if there were any differences between conditions where the animals received light sessions first or dark sessions first. The animals in both sets received all the dose levels of dA sulphate. Equal volume saline injections (1 mg/kg body weight) containing the respective quantities of dA were injected intraperitoneally on each of the 13 experimental days. Injections were given 15 min before data collection began. The subject spent the last 5 min of this period in the recording chamber adapting to the ex-

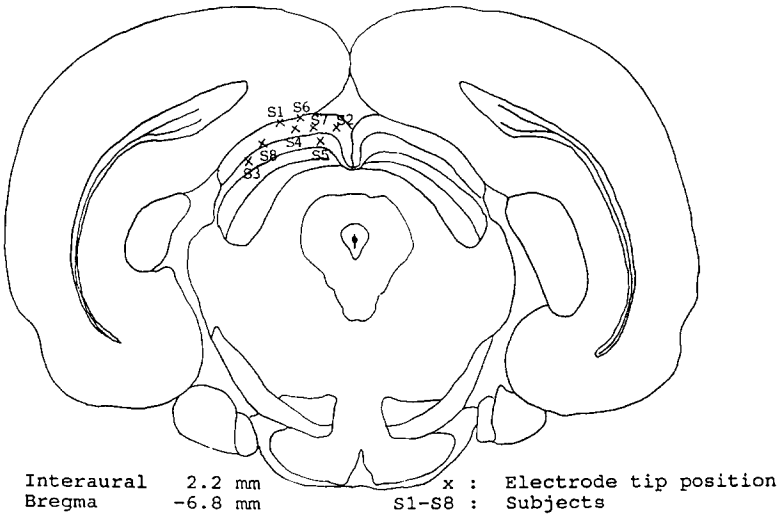

FIG. 1. Electrode placements in the superior colliculus of the subjects [drawing of the coronal section through the superior colliculus was adapted from Paxinos and Watson (21)].

isting sensory conditions.

Each session was 40 min in duration with electrophysiological response measures recorded in 5-min intervals, and light and dark sensory conditions were alternated over daily sessions throughout the experiment. The data from the last eight days, four in the light and four in the dark, were analyzed. Only data from the last six 5-min intervals were analyzed to eliminate potential confounding by any response to attaching the plug and cable assembly, and to allow for habituation to the test environment.

Histological Procedure

After all of the data were collected, each subject was euthanized with Nembutal overdose. It was then perfused with normal saline followed by 10% formalin-saline. For histological analysis, the brains were removed and sections of the tissue were cut at 40 μm. Every fifth section was mounted on a slide and stained with cresyl violet. The SC electrode placements were verified under light microscope by visual inspection.

RESULTS

Visual inspection of the brain sections showed that the electrode tips were localized within the layers of the SC (see Fig. 1).

Analysis of variance (ANOVA) was used in evaluating the data. A mixed two-factor ANOVA was initially performed to determine the response effects due to the sequence of light-dark alternating test sessions (LD/DL sequence). Since no significant differences were found for this factor in the arousal index (AI) data the scores in the LD and DL groups for the respective dose levels were combined. Analysis of the AI data, showed that the effect due to the illumination condition was significant, $F(1,7) = 62.28$, $p<0.001$, with the AI being greater in the dark than in the light (see Fig. 2). There was no significant effect for the dose factor. The interaction effect between illumination and dose was also not significant.

Since the effects due to LD/DL sequences for the locomotor activity data also proved to be nonsignificant, the scores in the two groups were pooled for the light and dark conditions. ANOVA for the locomotor activity data revealed that there was no main effect for the illumination factor. The main effect due to the dose levels of dA, however, was significant at the 0.001 level, $F(3,21) = 8.4$, as was the dose and illumination interaction effect, $F(3,21) = 11.3$ (see Fig. 3). A post hoc analysis of the scores showed that the activity level in the light condition

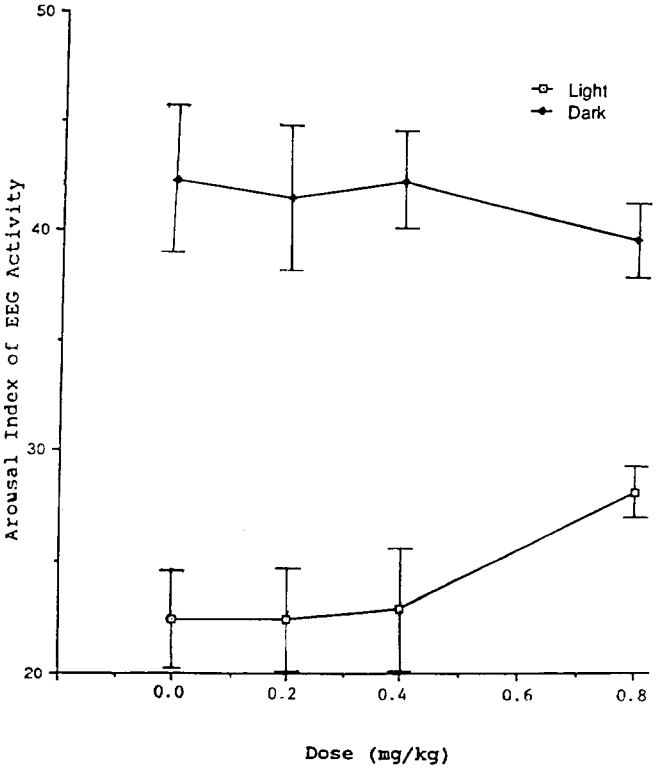

FIG. 2. Mean arousal index (AI) of EEG activity in light and dark conditions.

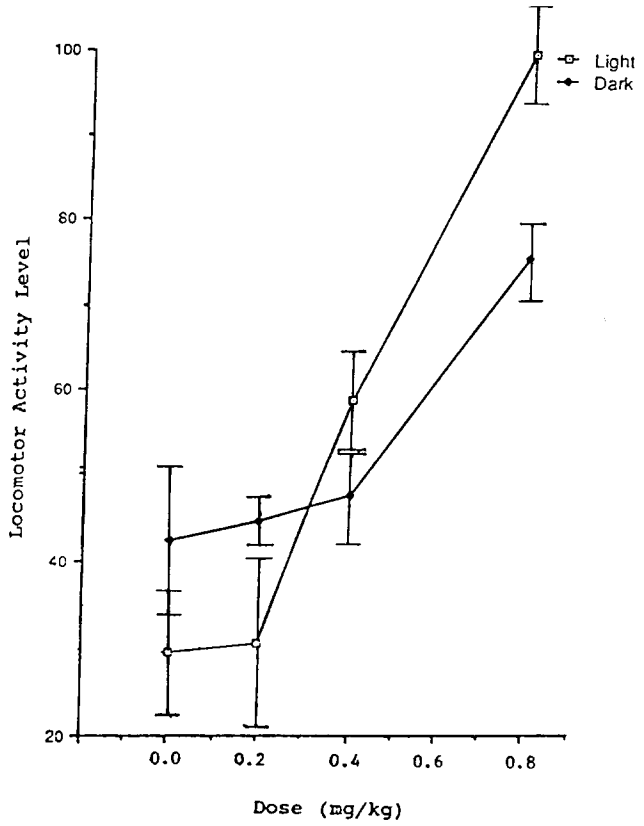

FIG. 3. Mean locomotor activity in light and dark conditions.

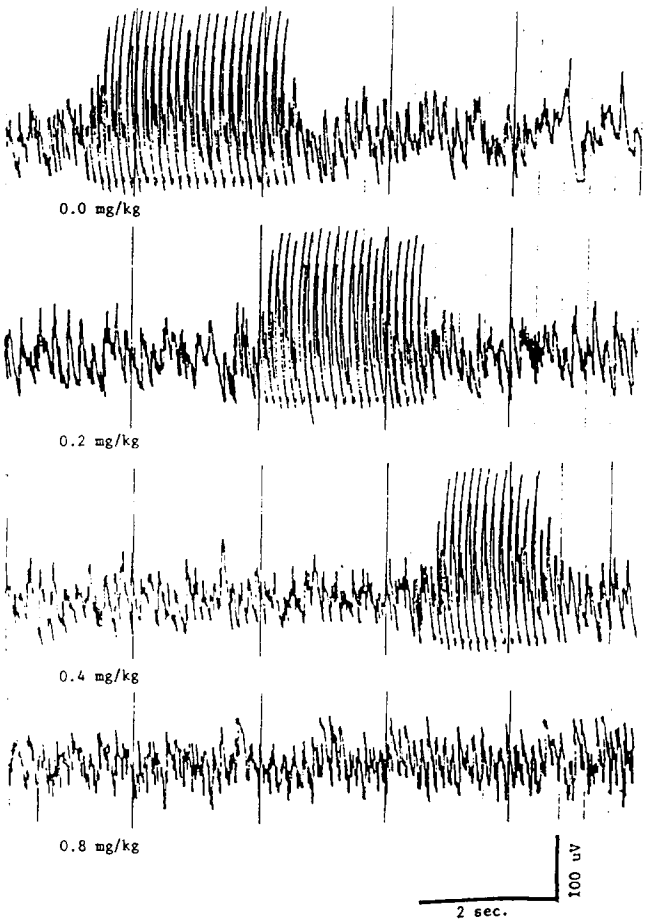

FIG. 4. EEG recording of superior collicular activity in response to d-amphetamine.

under 0.8 mg/kg of dA was significantly greater that those in the 0.0 and 0.2 mg/kg levels under both light and dark. Activity under 0.8 mg/kg of the drug was also significantly greater than those under 0.0 and 0.2 mg/kg of dA in the light.

The fact that no dose effect was found in the AI data may have been due to the sampling pattern (two s per 15-s interval) of the EEG output by the computer. Upon examination of the data output recording on paper on the EEG machine, it was observed that the SC emitted bursts of high-voltage alpha waves of approximately 600 μV and 10 Hz (see Fig. 4). These waves occurred in two- to four-s interval bursts after which the EEG would return to lower voltage waves (approximately 150 μV) which predominated the EEG activity. For purposes of discussion, these high-voltage alpha waves will be referred to as collicular spike activity. It was also noticed that the spike activity was absent when 0.8 mg/kg of d-amphetamine was given (see Fig. 4). Thus a count of the total number of seconds the spike activity occurred during a testing session was made.

ANOVA comparing the effects of illumination and dose levels on the amount of spike activity was then performed. The results indicated that the main effect due to dose levels was significant, $F(3,21) = 17.24$, $p<0.001$. The main effect due to illumination and the interaction effect of illumination with dose were not significant. Post hoc analysis showed that the amount of spike activity under 0.8 mg/kg of d-amphetamine in light and dark was significantly less than those in the 0.0 and 0.2 mg/kg levels in light and dark (see Fig. 5).

DISCUSSION

Results showed that the AI of the SC under light conditions was significantly lower than that in dark conditions. It would seem that light attenuated the AI. The decrement in AI in response to light supported the present study's hypothesis. The fact that light alters the electrophysiological activity of the SC has also been demonstrated by Siminoff, Schwasson and Kruger (27). Recording from units of cell aggregates or "receptive fields" of the SC, these researchers found that as long as light stimuli were projected onto the visual system, spontaneous activity of these units was inhibited. Since rats have been found to be more active in the dark than in the light [e.g., (24)], it is plausible that light acts to reduce the AI of the SC thereby producing hypoactivity. For diurnal animals, however, light may serve to increase the AI of the superior colliculus causing hyperactivity in the organism. This hypothesis for the diurnal animal remains to be tested.

The present study also hypothesized that administration of dA would affect the AI. However, in both light and dark conditions, the effect due to the dose levels of dA on collicular activity was not statistically significant. Nevertheless, it should be pointed out that at 0.8 mg/kg of dA, the level of the AI showed a slight decrease. Conversely, the AI in the light showed an increment at this level of the drug. It would be interesting to see how the levels of the AI respond to higher doses of the drug.

The change in collicular activity due to the effects of dA may have been inadequately detected by the computer possibly because of the amount of sampling time. Visual examination of the electrophysiological recording output on paper showed that

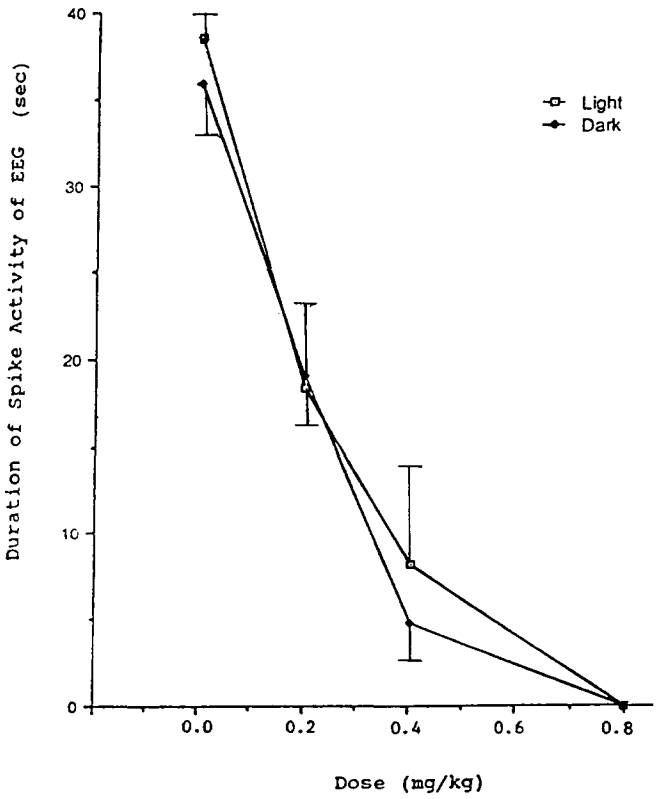

FIG. 5. Mean collicular spike activity in light and dark conditions.

there were spontaneous emissions of high-voltage waves which lasted for an average of two- to four-second intervals. These were arbitrarily referred to as spike activity. Results of the amount of spike activity occurring in a single testing session revealed that in the placebo conditions, the duration was relatively high, occurring for approximately 30 to 40 s within a 30-minute recording session. The amount of spike activity was related to the dose level of dA administered; as the drug dose increased, spike activity decreased until at 0.8 mg/kg, the high-voltage activity was nonexistent. At this stage, it is difficult to determine the cause of the emission of the high-voltage waves; a number of factors or specific behavioral patterns could have triggered off such EEG activity. This phenomenon could be studied in greater detail and precision.

The effect of dA on the animals' overt behavior is seen in the increase in locomotor activity as a function of the dose level of the drug. Furthermore, the effect of the drug interacts with illumination conditions to affect activity. This is consistent with previous findings that dA produces larger changes in locomotor activity in light conditions and less obvious changes in dark conditions and lends further support to the notion that the drug exerts its effects on locomotor activity by reducing the effect of light upon the arousal mechanism (1,13).

It is possible that dA exerts its influence on locomotor activity via the SC. It has been found that fiber connections from the SC terminate in the reticular formation, particularly the nucleus reticularis gigantocellularis, nucleus reticularis pontis caudalis, and nucleus reticularis pontis oralis (15). Anderson, Yoshida and Wilson (4,5) demonstrated that these nuclei exerted a strong influence on the descending reticulospinal pathways especially those to the muscles of the limbs. From this, it is suggestive that the SC has an influence upon an organism's movement and locomotor activity. Isaac (10) and, later, Lynch and Crain (18) proposed that this influence is inhibitory in nature, and that upon enucleation of the SC, release from inhibition occurs resulting in increased activity. This theory has received wide support. Foreman, Goodale and Milner (7) by lesioning the SC in rats, found increases in locomotor activity in the open field, head-raising, rearing, and sniffing when compared to sham-controls. Similar effects of hyperactivity upon collicular damage have been documented by other researchers (28,22).

There is ample evidence to show that dA acts on the SC to produce hyperactivity [e.g., (6,26)]. Although not well understood, results of the collicular spike activity in the present study provides rudimentary evidence at the electrophysiological level to show that dA acts on the superior colliculus.

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