protein, and, since ACTH increases the adrenal uptake of ^{45}Ca (*17*), calcium may be responsible for the activation of the transfer enzymes by ACTH. Conceivably, this may be the initial or early step whereby ACTH stimulates adrenal growth, since the early in vitro effect of ACTH on leucine incorporation in rat adrenal sections appears to depend upon calcium. Since cyclic AMP (exogenous) also has calcium-dependent stimulatory effects on adrenal protein synthesis, it is possible that this nucleotide is ultimately responsible for the calcium-mediated stimulation of adrenal protein synthesis by ACTH. However, attempts to show that cyclic AMP enhances ^{45}Ca uptake by the adrenal have not been successful (*17*).

While the above findings suggest that calcium may (at least partly) mediate the effect of ACTH on adrenal growth, it should not be construed that calcium mediates the steroidogenic effect of ACTH. In other experiments (*16*), calcium *alone* stimulated adrenal protein synthesis, but had no effect on steroidogenesis unless ACTH or cyclic AMP was present. Thus, if ACTH induces the synthesis or activation of a metabolically labile, steroidogenic protein, the synthesis of this protein may require calcium to maintain optimum protein synthesis, but calcium itself does not appear to be the inducing factor.

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Melatonin and Abnormal Movements Induced by L-Dopa in Mice

Abstract. *Melatonin has blocked adventitious movements induced by* L-*dopa in intact mice. It has reversed the adventitious turning to the right, and it has induced running to the left in mice receiving* L-*dopa after a lesion in the right caudate nucleus.*

Administration of melanophore stimulating hormone (β-MSH) to patients with Parkinsonism darkened their skins and aggravated their tremor (*1*). The assumption was pursued that the darkening skin deprived the brain of neurotransmitters by sequestering their precursors (*2*). This led to control of Parkinsonism first with D,L- (*1*) and thereafter with L-dopa (*3*). We were much less impressed by therapy with an antagonist of β-MSH, melatonin (*4*).

At the peak of therapy with dopa, abnormal movements emerged (*1, 3*). These have been thought of [see (*4*)] as perhaps related to the diminution of cerebral serotonin by L-dopa (*5*). In turn, the concentration of serotonin in the brain was increased by administering melatonin (*6*).

The present experiments are not aimed specifically at treatment, but rather they serve to (i) illustrate a radical alteration of the effects of L-dopa by melatonin and (ii) settle, perhaps, some notions about treating Parkinsonism.

More than 500 intact male Swiss albino mice of the Hale-Stoner strain were tested. Under specified conditions (*7*), these mice developed abnormal movements from L-dopa (Nutritional Biochemicals Corp.), whereas those of another strain were highly resistant (*7*). In addition, 300 Hale-Stoner mice were included after partial suction of the right caudate nucleus. [The operations and the pretesting were performed according to Lotti (*8*)]. Immediately after operation, these animals turned their heads (and often their bodies and tails) to the right and retracted their limbs on the right under their bodies while extending those on the left (*9*). Over the next 10 days, they gradually resumed mobilities and postures similar to intact mice. Thereafter, apomorphine (Merck & Co.) given intraperitoneally (2 μg per gram of body weight) reproduced the adventitious turning and most of the other signs observed postoperatively. Animals previously tested with apomorphine were included in further experimentation a week later.

For the further experimentation, nonlethal doses were selected. The LD_{50} (lethal dose, 50 percent effective) of the orally administered melatonin (Sigma Chemical Co.; Regis Chemical Co.) was shown by probit analysis to be roughly 2.4 mg/g after 24 hours. The LD_{50} of melatonin given intraperitoneally was approximately 1 mg/g. Intraperitoneal injections of this hormone (0.4 to 0.8 mg/g) drastically diminished the mortality from oral doses of L-dopa [3 mg/g (*7*)], whereas orally administered melatonin failed to do so.

In intact mice, oral L-dopa (3.0 mg/g) induced the adventitious movements discussed earlier (*7*). Administration of melatonin (0.4 to 0.8 mg/g intraperitoneally or 1.0 to 2.5 mg/g orally), either together with L-dopa or 30 minutes prior to it, blocked the hypermobility while lessening piloerection and salivation (*7*). The duration of these effects appeared dependent upon the dose of melatonin. In the operated animals, furthermore, special combinations of melatonin with L-dopa induced the additional phenomenon discussed below. This is partially illustrated in Fig. 1.

Given alone to operated mice, L-dopa (0.2 to 0.6 mg/g) duplicated the turning to the right and often the other signs evoked by apomorphine, although L-dopa induced more hypermobility again (*7*). The apomorphine-like signs of L-dopa could be reversed by combinations with melatonin given either orally (each 1.0 mg/g) or intraperitoneally (each 0.4 mg/g). These combinations changed the effects of L-dopa. Instead of merely turning to the side of the lesion, the animals turned to the left and ran in that direction for ½ hour or longer. These specific combinations seemed critical to the evolution of the full phenomenon, but mere turning to the right could be blocked by other combinations of dopa with melatonin (Table 1), as was the case with the adventitious movements of intact mice.

In man, administration of L-dopa has markedly increased the excretion

Table 1. Behavior of operated animals receiving melatonin and L-dopa. The doses are expressed as milligrams per gram of body weight; → is turning right; ←, turning left; ⇇, turning and running to left; 0, no turning; S, slight; M, marked; and I, immobile.

Intraperitoneal					Oral				
Melatonin	L-Dopa	Animals (No.)	Turning	Activity	Melatonin	L-Dopa	Animals (No.)	Turning	Activity
	0.2	18	→	S		1	5	→	S
	.3	15	→	S		1.5	5	→	S
	.4	30	→	M	1		5	0	I
	.6	20	→	S	1.5		5	0	I
0.4		7	0	I	1	1	5	⇇	M
.6		5	0	I	1	1.5	5	0	I
.3	.3	5	0	I	1.5	1.5	5	0	I
.4	.2	8	←	S					
.4	.4	38	⇇	M					
.6	.4	5	←	S					

of labile methyl groups (*3*), while in mice the cerebral concentration of the final methyl donor, *S*-adenosyl methionine, was lowered by L-dopa (*10*). Dopac [3,4-dihydroxyphenylacetic acid (Nutritional Biochemicals Corp.)] is a methyl acceptor which is not expected to generate neurotransmitter substances. Administration of dopac had blocked abnormal movements induced by L-dopa under specified conditions in intact mice (*7*). When this acid was now given by itself (0.2 to 2.0 mg/g) or with melatonin (0.4 mg/g) to operated animals, it produced apparent sedation but not turning or running. Intraperitoneal injections of dopac (0.2 mg/g) 30 minutes prior to the intraperitoneal injection of L-dopa (0.4 mg/g) blocked the turning to the right without inducing hypermobility or running. Doubling the dose of dopac delayed the onset of both turning to the right and hypermobility. These did emerge eventually, however, and were more intense than when L-dopa was given alone. Since dopac has failed to induce turning and running to the left, it did not seem to be a substitute for L-dopa in evoking this particular phenomenon.

Groups of five or six intact animals were injected thereafter intraperitoneally with one of the following solutions: (i) water for injection (0.02 ml/g); (ii) L-dopa (0.4 mg/g); (iii) melatonin (0.4 mg/g); or (iv) L-dopa plus melatonin (each 0.4 mg/g). One-half hour later, the mice were killed [between 11:30 a.m. and 12:30 p.m. (*11*)], and their brains were rapidly frozen in liquid nitrogen. The brains were analyzed for 5-hydroxyindoleacetic acid (5-HIAA), for serotonin (5-HT) (*12*), and for dopamine (DA) (*13*). The results are summarized in Table 2.

These analyses showed that melatonin tended to increase the cerebral levels of serotonin while dopa, given with or without melatonin, had increased those of dopamine. To determine whether increasing these neurotransmitters was sufficient for the full effect, we attempted in operated animals to (i) duplicate the turning and running with tryptophan, a precursor of serotonin (*14*), instead of with melatonin; and (ii) duplicate this behavior by substituting dopa with apomorphine, an analog of dopamine (*15–17*).

Coadministration of melatonin (0.4 mg/g) and apomorphine (0.25 to 2 mg/g) to seven groups of five animals each rarely blocked the turning to the right, and it did not cause running. Slight turning to the left was induced only when the melatonin was injected 5 minutes before giving 0.5 μg of apomorphine per gram intraperitoneally, but not otherwise. Neither the intensity of the turning to the left nor the running induced by melatonin plus dopa was duplicated with apomorphine. In addition, injections of L-tryptophan (Nutritional Biochemicals Corp.; 0.4 to 1.0 mg/g) to six groups, either together with L-dopa (0.4 mg/g) or 30 minutes earlier, induced only slight turning of the head to the left in some animals, but no running. Indeed, all animals now remained immobile and apparently sedated.

Melatonin did not decrease the uptake of L-dopa by the brain, as surmised by the cerebral levels of dopamine generated from L-dopa. For the same reason, melatonin did not seem to act as a

Table 2. Mean ± standard deviation of dopamine, serotonin, and 5-hydroxyindoleacetic acid (in micrograms per brain) 30 minutes after intraperitoneal injections of the chemicals listed. The comparisons of the following combinations showed $P < .01$: 1A versus 2A, 1A versus 4A, 2A versus 3A, 3A versus 4A, 1B versus 3B, and 2B versus 3B; 1A versus 3A showed $P < .05$.

Animals	Dopamine (A)	Serotonin (B)	5-Hydroxyindoleacetic acid (C)
1. Control	0.45 ± 0.01	0.22 ± 0.01	0.093 ± 0.003
2. L-Dopa	2.09 ± .16	.19 ± .02	.084 ± .01
3. Melatonin	0.49 ± .01	.32 ± .02	.087 ± .003
4. Melatonin + L-dopa	2.06 ± .14	.26 ± .02	.093 ± .009

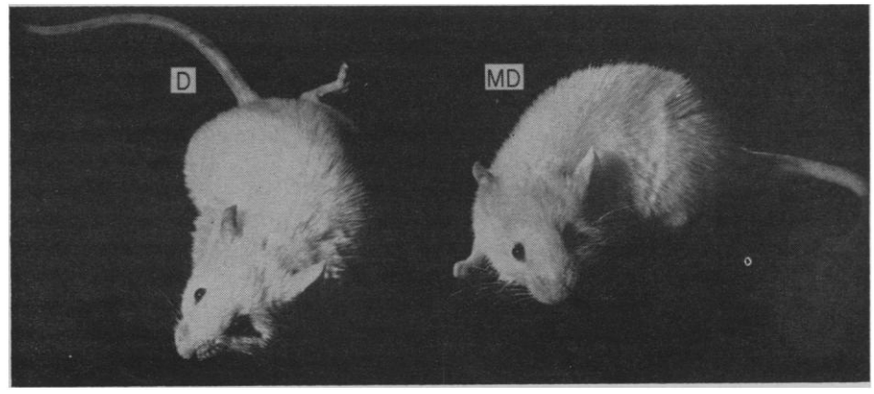

Fig. 1. Three weeks prior to the experiment, both mice had a partial ablation of their right caudates. Mouse D received L-dopa and mouse MD melatonin and L-dopa (each 0.4 mg/g, intraperitoneally) 30 minutes prior to the experiment.

dopa decarboxylase inhibitor. It has elevated cerebral serotonin, but an effective precursor of cerebral serotonin has failed to induce the full phenomenon described here. Since both L-dopa and melatonin appeared essential for turning and running, competition of neurotransmitters for neuronal receptors does not appear to offer an adequate explanation. We cannot interpret these findings at present in molecular or physiological terms. We can, however, determine their possible relevance to the involuntary movements induced by L-dopa in Parkinsonism.

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Eye-Head Coordination in Monkeys: Evidence for Centrally Patterned Organization

Abstract. *Eye-head coordination was investigated by recording from the neck and eye muscles in monkeys. The results show that (i) during eye-head turning, neural activity reaches the neck muscles before the eye muscles, and (ii) all agonist neck muscles are activated simultaneously regardless of the initial head position. Since overt movement of the eyes precedes that of the head, it was concluded that the central neural command initiates the eye-head sequence but does not specify its serial order. Furthermore, it was determined that the compensatory eye movement is not initiated centrally but instead is dependent upon reflex activation arising from movement of the head.*

Complex coordinated movements entail a temporally patterned sequence of motor and neuromuscular events. In order to better understand these sequences and their relation to each other, we have used behavioral and physiological methods to investigate the coordination of eye and head movement in monkeys. From earlier work on man (*1*) and from our observations of monkeys, we know that the appearance of a target in the visual field is usually followed by a saccadic eye movement directed toward the target, and then, after a latency of 25 to 40 msec (in monkeys), by a head movement in the same direction. Since the eyes move first and with a high velocity, their lines of sight typically reach the target and fixate it while the head is still moving toward it. For the duration of the head movement the eyes maintain their fixation by performing a rotational movement which is counter to that of the head and compensates for it—hence the name compensatory eye movement (*2*).

Thus, the act of directing the eyes and head to a target is composed of a fairly rigid pattern of events. A visual saccade carries the fovea near or to the image of the target. This is followed by a head movement in the same direction, which is accompanied by a compensatory eye movement.

We have taken advantage of this well-defined sequence of motor events to investigate, first, the spatial and temporal patterning of the neural command to the various muscles involved in eye-head turning. In a second set of experiments we sought to determine if the temporal sequence of saccade followed by compensatory eye movement is centrally programmed or, alternatively, if the compensatory movement is the result of a reflex activation induced by head turning. And, as a step toward eventually providing a quantitative description of the neural and behavioral aspects of eye-head coordination, we have paid close attention to the reliability of visually triggered eye-head movements.

Four adult monkeys (*Macaca mulatta*) were used in these experiments. The animals were trained to turn correctly and to identify a 1-degree luminous target which appeared randomly at different horizontal positions in their field of vision. A lightweight apparatus was attached to the animal's head to record movements in the horizontal and vertical planes. Eye movements were recorded with silver–silver chloride pellet electrodes implanted in the orbital bone (*3*). Electromyographic (EMG) activity associated with horizontal eye movements was recorded from the lateral rectus muscle, along with the activity from the seven neck muscles involved in horizontal head rotation (trapezius, cleido-occipitalis, splenius capitis, longissimus capitis, complexus, obliquus capitis posterior, rectus capitis dorsalis major) (*4*). During the recording sessions the monkey's trunk was restrained.

The results show that, after the onset of the target light, the first event to take place is invariably a synchronous burst of EMG activity in all agonist neck muscles, which is usually followed by a 20- to 30-msec pause (Fig. 1). Concurrently, EMG activity is suppressed in all the antagonists, and, as Fig. 2 shows, both these events occur with a latency of approximately 160 msec.

That the bursts appeared simultaneously in all the agonists regardless of the initial head position of the animal is quite remarkable. We found, however, that the amplitude and duration of the bursts were dependent upon the starting position and the extent of the intended head movement.

The second EMG event to take place is an eye muscle burst, which always occurs approximately 20 msec after the beginning of neck muscle activity (Fig. 2).

Taken together, these EMG results indicate the spatiotemporal characteristics underlying sequentially ordered eye and head movement. The neural commands are, in fact, delivered simultaneously to all neck muscles and shortly thereafter also to the eye muscles. However, the overt sequence of movements that results from these commands does not reflect this order insofar as the head movement actually oc-