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24. We examined four animals in stage 6. Fast (short sarcomere) fibers ranged from 41 to 64 percent (mean, 56 percent) in the cutter and from 6 to 32 percent (mean, 21 percent) in the crusher.
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26. The regenerating claw is enclosed in a tough, transparent capsule and cannot be used until after the next molt.
27. This experimental group began with 12 animals. However, there was a higher than normal loss because animals often lost a claw during the molt to stage 5 or 6. Of the seven animals that were successfully reared, four had left crusher claws and three had right crusher claws. While this procedure eliminates shortening of the closer muscle, it probably does not interfere with isometric contraction; the influence on normal motor activity, if any, is not known.
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Rebound Insomnia: A New Clinical Syndrome

Abstract. *Rebound insomnia followed the withdrawal of three benzodiazepine hypnotic drugs, each of which had been administered in a single nightly dose for only short-term periods. The intense worsening of sleep is attributed to the short duration of the action of these drugs. A hypothesis involving benzodiazepine receptors in the brain is proposed in which there is a delay or lag in replacement of endogenous benzodiazepine-like molecules after the abrupt withdrawal of exogenous drugs.*

We previously reported that a worsening of sleep was associated with the abrupt withdrawal of nonbenzodiazepine hypnotic drugs that had been administered in multiple nightly doses over a long period (*1*). We termed this condition drug-withdrawal insomnia (*1*) and considered it part of a general abstinence syndrome resulting from the withdrawal of depressant drugs and related to supersensitivity in the central nervous system (CNS) (*2*). We now describe a new clinical entity, "rebound insomnia," which consists of a marked worsening of sleep following the abrupt withdrawal of certain benzodiazepine drugs administered in only single doses nightly for short periods.

We have now analyzed data from six separate sleep laboratory evaluations of three benzodiazepine hypnotic drugs (*3–8*); three of these studies, one with each drug, were conducted in our laboratory (*3*, *6*, *7*). According to the data, an intense form of rebound insomnia occurs after the withdrawal of only a single nightly dose of certain benzodiazepine hypnotic drugs that had been administered for short and intermediate as well as long periods.

The study designs all included an initial placebo-baseline period, a short-, intermediate-, or long-term drug administration period, and a placebo-withdrawal period. Each study evaluated only one drug in a fixed dose. All subjects were insomniacs who were continuously monitored by electroencephalogram (EEG), electromyogram (EMG), and electrooculogram (EOG).

Kales *et al.* (*3*) evaluated triazolam (0.5 mg) in seven subjects according to a 22-night protocol including four placebo-baseline nights (the first for adaptation and the next three for baseline measurements), 2 weeks of nightly drug administration, and four placebo-withdrawal nights (Table 1). Although short-

Table 1. Effects of benzodiazepines on the induction and maintenance of sleep. Data are means and standard errors. The total time of recording each night was 8 hours. All statistical comparisons are with the baseline data; the Dunn multiple comparison *t*-test was used for the analyses.

Condition	Day	Time awake (minutes)			Wakings (No.)
		Latency to sleep	After sleep onset	Total	
		Triazolam (0.5 mg) (3)			
Baseline	2 to 4	61.4 ± 9.7	32.8 ± 6.1	94.2 ± 11.8	20.4 ± 2.3
Drug administration					
Short-term	5 to 7	35.5 ± 3.2	16.2 ± 1.9*	51.7 ± 3.5†	14.4 ± 1.0†
Intermediate-term	16 to 18	49.0 ± 4.0	29.2 ± 4.8	78.2 ± 5.8	20.3 ± 2.2
Withdrawal‡	19 to 21	97.3 ± 13.8†	53.2 ± 9.0*	150.5 ± 19.3†	21.5 ± 2.2
		Flunitrazepam (1 mg) (6)			
Baseline	2 to 4	43.0 ± 4.2	33.7 ± 3.4	76.7 ± 5.6	26.3 ± 1.5
Drug administration	5 to 11	41.3 ± 2.1	33.6 ± 3.6	74.9 ± 4.5	19.9 ± 1.0†
Withdrawal	12 to 14	67.7 ± 9.0†	53.1 ± 10.0*	122.8 ± 14.2†	23.6 ± 1.8
		Nitrazepam (10 mg) (7)			
Baseline	2 to 4	39.6 ± 5.3	34.8 ± 3.1	74.4 ± 8.7	23.9 ± 2.0
Drug administration	5 to 11	26.4 ± 1.5*	20.1 ± 1.6*	46.5 ± 2.4†	17.5 ± 1.2
Withdrawal	12 to 14	38.5 ± 6.2	65.3 ± 13.1†	103.8 ± 15.4†	22.3 ± 1.9

*$P < .05$. †$P < .01$. ‡Only the first three nights of withdrawal were used in this analysis.

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term drug administration markedly improved both the induction and the maintenance of sleep, at the end of 2 weeks of drug administration, none of the measures remained significantly decreased from baseline. After the drug was withdrawn, sleep markedly worsened beyond baseline levels; sleep latency, time awake after the onset of sleep, and total time awake were all significantly increased from baseline values.

Triazolam (0.5 mg) has been evaluated in two additional studies. Vogel *et al.* (*4*) evaluated six subjects in a 14-night experiment consisting of four placebo-baseline nights, 1 week of nightly drug administration, and three placebo-withdrawal nights. Total time asleep significantly increased during drug administration ($P < .05$) and significantly decreased after drug withdrawal ($P < .01$). Roth *et al.* (*5*) studied eight subjects according to the 22-night protocol previously described. Sleep latency was significantly decreased with short-term ($P < .05$) and intermediate ($P < .01$) drug administration, and after the drug was withdrawn, the percentage of time awake after sleep onset significantly increased ($P < .05$).

Bixler *et al.* (*6*) has investigated the effect of flunitrazepam (1 mg) on 12 subjects according to the 14-night protocol (Table 1). Even though this dose was not effective for either inducing or maintaining sleep, sleep worsened significantly after the drug was withdrawn.

Soldatos *et al.* (*7*) evaluated nitrazepam (10 mg) in four subjects according to the same 14-night protocol (Table 1). Drug administration significantly decreased sleep latency, time awake after sleep onset, and total time awake. Following drug withdrawal, time awake after sleep onset and total wake time increased significantly over baseline levels.

Nitrazepam in a 5-mg dose was evaluated by Adam *et al.* (*8*). This study included 10 weeks of drug administration that were preceded by six placebo-baseline nights and followed by six placebo-withdrawal nights. Total time of sleep improved significantly on drug nights early in the study ($P < .05$) as well as on later ones ($P < .01$). Drug withdrawal significantly increased total time awake ($P < .05$).

In all of the studies described (except that of flunitrazepam), the drug clearly suppressed rapid-eye-movement (REM) sleep. However, after the drug was withdrawn, there was no REM rebound above baseline levels in any of the studies. Similarly, stages 3 and 4 sleep were generally decreased with drug administration, and drug withdrawal resulted in a return only to baseline levels.

The six studies of triazolam, flunitrazepam, and nitrazepam discussed in this report represent all of the known sleep-laboratory studies conducted to date in which sufficient data are available to thoroughly evaluate the drug administration and withdrawal periods. Drug withdrawal in each of these studies was accompanied by a significant worsening of sleep compared with both baseline and drug administration nights. In addition, worsening of sleep occurred regardless of the efficacy of the drug just before it was withdrawn. For example, although the data from the three triazolam studies indicated that the drug was effective with short-term use, the results of one of these studies showed that most of the drug's effectiveness was lost by the end of 2 weeks of continuous use (*3*). Even though the effectiveness of triazolam varied in these three studies, rebound insomnia occurred each time it was withdrawn.

In previous studies, we have demonstrated that the abrupt withdrawal of REM-suppressing hypnotic drugs that have been administered in multiple nightly doses over long periods severely worsens sleep (*1*). We attributed this drug-withdrawal insomnia to several possible factors: patients' apprehension about their ability to sleep without the drug, the disruption of their sleep by the more intense and frequent dreaming associated with the marked rebound in REM sleep after the withdrawal, and a phenomenon of overcompensation of CNS excitability, after withdrawal of the CNS depressant drug. However, rebound insomnia has not been noted after the withdrawal of nonbenzodiazepine hypnotic drugs that have been administered in a single nightly dose for short-, intermediate-, and even long-term use (*9*).

In order to explain the intensity and severity of rebound insomnia found in each of the benzodiazepine studies reviewed here, in which only single doses of the drugs were administered nightly, we have used findings on the metabolism and pharmacokinetics of these drugs as well as new information concerning the presence of specific benzodiazepine receptors in the brain (*10*). The intense rebound insomnia that follows the withdrawal of triazolam, flunitrazepam, and nitrazepam is attributed to the much shorter duration of action of these drugs (*11*). Conversely, the absence of rebound insomnia after such benzodiazepines as flurazepam and diazepam are withdrawn is attributed to their having active metabolites with long half-lives (*12*).

The discovery of benzodiazepine receptors in the brain suggests the presence of endogenous benzodiazepine-like molecules whose production would be regulated by concentrations of the circulating molecules or a feedback mechanism. Production of endogenous benzodiazepine-like molecules would be decreased if active exogenous benzodiazepine drugs or metabolites were introduced. We hypothesize that abrupt withdrawal of those benzodiazepine drugs with a relatively short duration of action results in an intense form of rebound insomnia because of a lag in the production and replacement of endogenous benzodiazepine-like compounds. However, when benzodiazepines with long-acting metabolites are withdrawn, effects on the benzodiazepine receptors are less abrupt because the endogenous benzodiazepine-like compounds may be partially restored before the active metabolites of the exogenously administered drugs are completetly eliminated.

This model does not preclude the possibility that rebound insomnia may develop in response to benzodiazepines with long-acting metabolites that are taken for lengthy periods in high doses. The ability to produce endogenous benzodiazepine-like compounds within the time these long-acting metabolites are eliminated may be a function of dosage and the duration of drug administration.

It is possible that an analog of rebound insomnia occurs when certain short-acting benzodiazepine tranquilizers taken during the day are abruptly withdrawn. As the duration of action of the drug is exceeded, an increase in anxiety above baseline levels may occur, a condition that we term "rebound anxiety."

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Prolactin Binding Sites in the Rat Brain

Abstract. *The principles of the competitive-binding assay were used in conjunction with light microscopic radioautography to demonstrate specific prolactin binding sites localized on ependyma of the rat choroid plexus, a previously unknown prolactin target tissue.*

The competitive-binding assay is routinely used to detect the presence of specific, hormone binding sites in vitro. The technique is based on the principle that radioactively labeled and unlabeled hormone compete equally for receptor sites; specific binding is defined as the difference between bound hormone in samples receiving labeled hormone alone (experimental) and those receiving labeled hormone in the presence of an excess of unlabeled hormone (control). Recently, the technique has been applied in combination with radioautography to determine the distribution of specific protein hormone binding sites in vivo (*1–3*). Utilizing the principles of competitive binding in conjunction with light microscopic radioautography, we have localized specific prolactin binding sites on ependyma of the choroid plexus in the rat brain.

Adult, sexually mature male (200 to 250 g) and female (180 to 220 g) Wistar rats were anesthetized with an intraperitoneal injection of 35 mg of chloral hydrate per 100 g of body weight. Experimental rats (two male and two female) received an intracardial injection of ovine ^{125}I-labeled prolactin (4.48×10^6 dis/min) (*4*), and control rats (two male and two female) received an identical dose of ^{125}I-labeled prolactin plus a 500-fold excess (830 μg) of unlabeled ovine prolactin. Animals were perfused through the left ventricle with Ringer's lactate and then with Bouin's fixative. All male rats and one experimental and control female were perfused 5 minutes after hormone injection, while the remaining females were perfused 15 minutes after hormone injection.

Sections (4 μm thick) embedded in paraffin were cut from corresponding brain regions of experimental and control animals and were mounted on the same slide, stained with hematoxylin and eosin, and coated with Kodak NTB2 emulsion (*5*). Radioautographs were exposed for 12 to 18 days and were then developed for 6 minutes in Kodak D-170. Additional nonradioactive brain tissue (from one male and one female) were processed in an identical manner to determine background counts and to confirm the absence of radioautographic artifacts.

Quantitative comparisons of intensities of radioautographic reactions were carried out by counting silver grains per frame at a magnification of 1600 in a Zeiss photomicroscope. Each frame represented a unit area of 6960 μm^2. Eight frames per animal were counted over the choroid plexus and 25 frames per animal over the cerebral cortex, subfornical organ, subcommissural organ, median eminence (dorsal and ventral portions), hypothalamic ventromedial nucleus (VMN), dorsomedial nucleus (DMN), arcuate nucleus, and medial preoptic-anterior hypothalamic area (MPOAH) (*6*).

The brains of males and females (both those perfused 5 minutes and those perfused 15 minutes after hormone injection) exhibited similar results in all regions examined. The data in Table 1 summarize the results.

The strongest radioautographic reaction, that is, the greatest concentration of [^{125}I]prolactin, was found over the ependyma of the choroid plexus of ex-

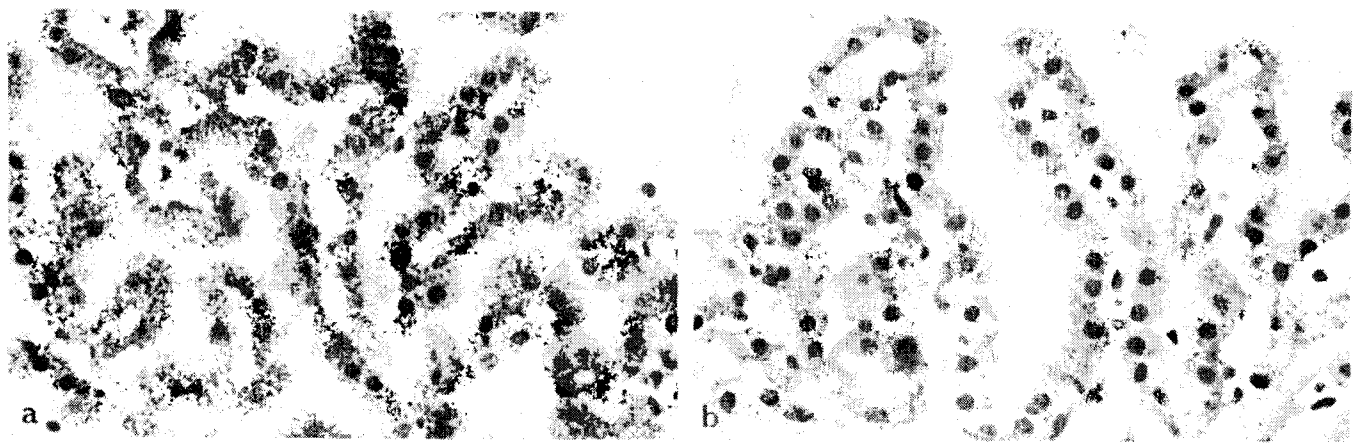

Fig. 1. Radioautographs of the rat choroid plexus after (a) injection of [^{125}I]prolactin (experimental), and (b) injection of [^{125}I]prolactin plus a 500-fold excess of unlabeled prolactin (control). A reduction of 81 percent in the number of silver grains occurs over ependyma of control tissue (×400).

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