

Clinical and Physiological Consequences of Rapid Tryptophan Depletion

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We review here the rapid tryptophan depletion (RTD) methodology and its controversial association with depressive relapse. RTD has been used over the past decade to deplete serotonin (5-hydroxy-tryptamine, or 5-HT) in humans and to probe the role of the central serotonin system in a variety of psychiatric conditions. Its current popularity was stimulated by reports that RTD reversed the antidepressant effects of selective serotonin reuptake inhibitors (SSRIs) and monoamine oxidase inhibitors (MAOIs) in remitted patients with a history of depression but not in patients treated with antidepressants which promote catecholaminergic rather than serotonergic neurotransmission (such as tricyclic antidepressants or bupropion). However, RTD has inconsistent effects in terms of full clinical relapse in depressed patients. Pooling the data from all published reports, patients who are either unmedicated and/or fully remitted are much less likely to

experience relapse (7 of 61, or ~9%) than patients who are recently medicated and partially remitted (63 of 133, or ~47%; although, the numbers here may reflect patient overlap between reports). Recently remitted patients who have been treated with non-pharmacological therapies such as total sleep deprivation, electroconvulsive therapy, or bright light therapy also do not commonly show full clinical relapse with RTD. We briefly review RTD effects in other psychiatric disorders, many of which are treated with SSRIs. There is accumulating evidence to suggest that RTD affects central serotonergic neurotransmission. Nevertheless, many questions remain about the ability of RTD to reverse the beneficial effects of SSRIs or MAOIs, or to induce symptoms in unmedicated symptomatic or asymptomatic patients.

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Serotonin (5-hydroxy-tryptamine, or 5-HT) is involved in many physiologic and behavioral systems and clinical

disease states. Much of the information available on the function of serotonin comes from pharmacological agents which mimic or amplify endogenous serotonergic neurotransmission, such as serotonin agonists or SSRIs. Historically, methods to functionally deplete serotonin have been problematic. Even previous approaches that have been moderately successful in experimental animals — such as the 5-HT synthesis inhibitor, parachloro-phenylalanine (PCPA); the neurotoxin 5-6-dihydroxy-tryptamine; or various lesions to the serotonergic raphe nuclei of the brainstem — have been non-specific, rife with secondary effects, or clearly inapplicable to humans (e.g., Engelman et al. 1967; Koella et al. 1968; Jouvret et al. 1967). The advent of rapid tryptophan depletion (RTD) as a method to deplete central serotonin has been met with enthusiasm because of its reversibil-

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ity, its relative lack of side effects, and its merit in elucidating the pathophysiology of various psychiatric conditions. This paper reviews the RTD methodology and its reported ability to induce a depressive relapse in euthymic patients, and other physiological and clinical effects.

RAPID TRYPTOPHAN DEPLETION

Availability of Tryptophan and Serotonin Synthesis

RTD is based on the current understanding of the 5-HT biosynthetic pathway, in which the first step is generally considered to be the rate-limiting one. The first step entails the hydroxylation of the amino acid TRP (by the enzyme TRP hydroxylase, or TRP-H) into the intermediate product 5-hydroxy-tryptophan, or 5-HTP. 5-HTP is then decarboxylated into 5-HT. Under normal conditions, TRP-H is about 50% saturated with TRP. Increasing or decreasing the availability of TRP correlates highly with the amount of 5-HT produced (e.g., Lin et al. 1969; Schaechter and Wurtman 1990). Because the body cannot synthesize TRP, reducing dietary intake of TRP reduces TRP levels in plasma and diminishes its transport from plasma into the brain.

In humans, TRP depletion is accomplished by consuming a TRP-free amino acid drink (e.g., Delgado et al. 1990; Biggio et al. 1974). This drink contains 15 amino acids in the same proportion as in human milk, except that TRP, aspartic acid, and glutamic acid are omitted (see Table 1). Within a matter of hours (4 to 12), plasma TRP declines to 10–50% of baseline levels (Delgado et al. 1990, 1991). Similar preparations deplete brain serotonin in animals (e.g., Gessa et al. 1974; Young et al. 1989; Biggio et al. 1974; Moja et al. 1991). The TRP-

depleting effects are enhanced with a low-TRP diet during the day preceding the challenge. To conduct studies according to double-blind, counterbalanced crossover design, subjects ingest either a TRP-free amino acid mixture or a "sham depletion" mixture (identical except that it contains 2.3 g TRP).

Tryptophan in Plasma, and Entry into Brain

Free versus bound TRP. Total TRP in plasma is comprised of free TRP plus (albumin-) bound TRP (McMenamy 1965). Only free TRP crosses the blood brain barrier (BBB), thus brain TRP levels are more accurately predicted by free TRP than total TRP levels (Tagliamonte et al. 1973). Factors such as plasma levels of non-esterified fatty acids (NEFA), which affect protein binding of TRP, may be relevant to RTD. NEFA displace TRP from its binding site on albumin (Curzon 1979), thus, potentially affecting the assignment of TRP to free vs. bound.

Other Amino Acids. Plasma levels of other amino acids are also important. TRP crosses over from plasma into the CNS via a specific BBB carrier (Oldendorf and Szabo 1976). All large neutral amino acids (LNAA: TRP, phenylalanine, leucine, isoleucine, tyrosine, and valine) (see Table 1) are transported by the same carrier, and thus compete with TRP for brain entry at these sites. Therefore, the TRP:LNAA ratio more accurately predicts brain TRP levels than absolute plasma TRP (e.g., Perez-Cruet et al. 1974; Fernstrom and Wurtman 1974; Fernstrom 1979).

Circadian and Dietary Influences. Plasma TRP levels exhibit diurnal or circadian variation in many species. In healthy human males after an overnight fast, plasma TRP reaches its minimum in the morning and peaks in the late evening (Wurtman et al. 1968). However, circadian influences are relatively minor compared to the influence of dietary intake. Foods that elevate brain TRP result in increased 5-HT synthesis (Fernstrom et al. 1979; Wurtman and Pardridge 1979). Paradoxically, this is accomplished by foods high in carbohydrate content, rather than high in protein and TRP. High-protein foods are rich in other amino acids that compete with TRP at the BBB, reducing TRP entry into brain. In part, high carbohydrate meals increase brain 5-HT by inducing insulin secretion, which traffics amino acids out of plasma and into tissue, leading to less competition for TRP uptake into CNS (Fernstrom et al. 1979).

Like a high protein meal, RTD introduces into plasma a bulk of amino acids that compete with TRP at the BBB. The amino acid load also stimulates protein synthesis, taking free TRP out of plasma and into new proteins (Moja et al. 1991).

Slowly depleting tryptophan by administration of long-term low-TRP diets to rats or humans (Moja et al. 1979; Lanoir et al. 1981; Delgado et al. 1989) reduces

Table 1. Composition of the 100 g Amino Acid Drink Typically Used in RTD Studies, and Aspects of its Amino Acid Contents

Amino Acid	Amount, g	Essential	LNAA	Hydrophobic
L-alanine	5.5			X
L-arginine	4.9	X		
L-cysteine	2.7			
Glycine	3.2			
L-histidine	3.2	X		
L-isoleucine	8.0	X	X	X
L-leucine	13.5	X	X	X
L-lysine	11.0	X		
L-methionine	3.0	X		X
L-phenylalanine	5.7	X	X	X
L-proline	12.2			
L-serine	6.9			
L-threonine	6.9	X		
(L-tryptophan)	(2.3)	X	X	X
L-tyrosine	6.9		X	X
L-valine	6.9	X	X	X

Abbreviation: LNAA = Large neutral amino acid.

plasma TRP below pre-diet baseline levels, although these reductions are relatively modest (e.g., to levels approximately 80% of baseline) compared with the effects of rapid tryptophan depletion (plasma TRP declines to 10–50% of baseline). Behavioral effects may be minimal or not sustained for the duration of the diet, possibly due to CNS adaptations.

RTD and 5-HT Release from Neurons

Early studies in rats showed that whole-brain 5-HT levels generally declined with RTD preparations of various kinds, but non-neuronal sources of 5-HT may have contaminated these findings. It is also unknown whether vesicular or cytoplasmic stores of neuronal 5-HT were preferentially affected. Since the firing rate of serotonergic raphe neurons appears to be unaffected by changes in TRP intake (Trulsson 1985), it is important to establish RTD's effect on neuronal release, and attempts to do so have been promising.

In vivo microdialysis measurements at projection sites of raphe neurons indicate changes in vesicular (i.e., action potential and Ca⁺⁺-dependent) 5-HT release. Systemic administration of 5-HT synthesis precursors such as L-TRP (Sharp et al. 1992) or 5-hydroxy-tryptophan (Gartside et al. 1992c) produced long-lasting and dose-dependent increases in 5-HT release in the ventral hippocampus and ventral hypothalamus, respectively. Decreases in electrically evoked 5-HT release in rat hippocampus followed systemic administration of valine, an LNAA (Gartside et al. 1992b). As an aside: in depressed patients and healthy subjects, administration of 30 g valine produced modest changes in mood, although not depressive relapse, compared to placebo (see Cowen et al. 1996). Administering an amino acid mixture also leads to decreased 5-HT release in rats, compared to saline vehicle (Gartside et al. 1992a), by about 45% of baseline.

In rats, an RTD-like mixture diminished 5-HT release measured by microdialysis in frontal cortex to about 50% of baseline, in a time course similar to the behavioral effects seen in analogous human studies (Bel and Artigas 1996). Other approaches have reported declines in hippocampal and striatal 5-HT with RTD-like preparations (Brown et al. 1998). It should be noted that 5-HT microdialysis studies are often performed under general anesthesia, in a state that is different from wakefulness or sleep, and state of consciousness is the single most prominent influence on the firing activity of the raphe (Jacobs et al. 1990; Fornal et al. 1994). Furthermore, substances such as SSRIs or fenfluramine are often added to the preparation in order to enhance 5-HT release to measurable levels. With current technology, levels of 5-HT release under baseline conditions are too low to be detected. At this point, it is difficult to ascertain changes in 5-HT levels in a state uninfluenced by 5-HT enhancing drugs.

RTD and the Neuroanatomy of the Serotonergic Raphe of the Brainstem

Due to its neuroanatomy and role in neurodevelopment, 5-HT is well positioned to modify most other neurotransmitter systems in the CNS. The cell bodies of the serotonin-producing raphe neurons are clustered along the midline of the brainstem and midbrain (fairly early-evolved brain structures). The projections of these neurons and hence the sites of potential 5-HT influence extend over every level of the neuraxis. These other systems could be affected or disrupted by a sudden drop in 5-HT release. In this way, the RTD paradigm might simply uncover other systems normally being modulated, modified, or otherwise regulated by 5-HT. For example, in neocortex as well as within hippocampal structures, serotonin receptors are found on other interneurons (Tork 1990). 5-HT by itself does not alter firing of quiescent spinal motor cord neurons, but it is believed to alter the response threshold to glutamate (Baumgarten and Grozdanovic 1995). Serotonergic projections are also known to facilitate the dopaminergic mesolimbic reward pathway, to inhibit the noradrenergic locus ceruleus system, and to inhibit release of acetylcholine in hippocampus and cortex (Baumgarten and Grozdanovic 1994).

Evidence that RTD Reduces 5-HT in Human Volunteers or in Animals

Plasma TRP. Over dozens of studies, the RTD paradigm produces a consistent and robust attenuation of TRP levels in plasma (see Tables 2, 3, and 4). Even when the specific composition of amino acid challenge itself is altered, plasma TRP falls. Lesser degrees of plasma TRP depletion are observed when smaller total amounts of amino acids are ingested (Moja et al. 1988, 1989). Larger amounts of amino acids not only produce lower nadirs of TRP in plasma, but also prolong the time to nadir (Moja et al. 1989).

Melatonin Secretion, CSF Levels of TRP and CSF Levels of Serotonin Metabolite, 5-Hydroxy-Indole Acetic Acid (5-HIAA). Serotonin is an intermediary product in melatonin synthesis. Compared to sham depletion, RTD significantly attenuated the secretion of melatonin across the night in eight of eight healthy volunteers (Zimmerman et al. 1993) (see Table 2). Levels of TRP in cerebrospinal fluid (CSF) and CSF levels of 5-HT metabolite 5-HIAA were significantly diminished within two hours of RTD ingestion and reached their nadir around six to twelve hours post-ingestion (Williams et al. 1999; Carpenter et al. 1998).

5-HT Synthesis Measured by Positron Emission Tomography (PET). Nishizawa et al. (1997) devised a PET method to estimate rates of serotonin synthesis, in

Table 2. Evidence that Different Preparations of RTD Deplete Central Serotonin, in Healthy Human Volunteers or in Animals

Measure of Interest	Reference	Subjects	% TRP Decline	Results and Comments
Plasma TRP, brain TRP, and brain 5-HT	Moja et al. 1988	28 male Wistar rats	(see results)	Rats were administered water or one of three mixtures of amino acids in differing strengths. The decrease in total plasma TRP, free brain TRP, brain 5-HT and brain 5-HIAA was proportional to the amount of amino acids ingested.
Brain 5-HT	Biggio et al. 1974	Male Wistar rats	~10%	Rats ingested either a 10.5g tryptophan-containing diet or a 10.3g tryptophan-free diet. Both diets also contained other nutrients in addition to amino acids. Brain 5-HT declined to 43% of baseline values, an effect that was maximal within two hours of ingestion.
5-HT release from neurons	Gartside et al. 1992a and b	Male Sprague-Dawley rats	n.r.	Rats were administered either vehicle, L-valine, or a mixture of amino acids. L-valine or amino acids (but not vehicle) led to decreases in 5-HT release from serotonergic raphe neurons at hippocampal projection sites. Measurements were taken in anesthetized rats.
5-HT release from neurons	Bel and Artigas. 1996	Male Wistar rats	n.r.	Amino acid mixtures comparable to those used in human RTD studies were administered to rats. The tryptophan-free amino acid mixture led to reduced 5-HT release from serotonergic raphe neurons in frontal cortex, but only in animals that had been pre-treated for two weeks with SSRI.
Cerebrospinal fluid (CSF) levels of TRP & 5-HIAA	Williams et al. 1999	2F, 4M	87%	Measured the levels of TRP and 5-HIAA in cerebrospinal fluid (CSF). In the hours following RTD, TRP in CSF declined similar to the TRP decline in plasma. CSF 5-HIAA levels also dropped compared to baseline, but to a lesser extent than plasma TRP did.
CSF levels of TRP & 5-HIAA	Carpenter et al. 1998	2F, 3M	85%	Measured CSF levels of TRP and 5-HIAA, as well as CSF levels of tyrosine and homovanillic acid (HVA). CSF TRP declined to 10% of baseline; CSF 5-HIAA declined to 24–40% of baseline. No effects were found for tyrosine nor HVA, suggesting specificity RTD's for the 5-HT system.
Secretion of melatonin	Zimmerman et al. 1993	4F, 4M	90%	All 8 subjects showed decreased melatonin measurements across the night. Here, the RTD challenge had been administered at 3pm (in contrast to 8 or 9 am in most studies).
Neuroendocrine effects	Coccaro et al. 1998	6M	79%	Measured the prolaction response to d-fenfluramine challenge. Pre-treatment with RTD attenuated the prolaction response.
Serotonin synthesis [measured by positron emission tomography (PET)]	Nishizawa et al. 1997	7F, 8M	F: 88% M: 73%	Rates of 5-HT synthesis were calculated from PET measurements before and after RTD. Baseline pre-RTD synthesis rates were much lower in females than males. In addition, following RTD, females' rates of 5-HT synthesis were depleted by a greater fraction compared with males (females: 1/40 th , males: 1/10 th).
EEG sleep	Bhatti et al. 1998	10M	79%	Administered 25g or 100g mixture at 3pm. 100g elicited a significant reduction of REM sleep latency in nighttime sleep recordings. 25g challenge also produced significant changes in REM sleep, findings consistent with a reduction in 5-HT release from neurons.

Abbreviations: TRP = tryptophan; 5-HT = serotonin; 5-HIAA = 5-hydroxy-indole acetic acid; EEG = electroencephalographic.
% TRP decline indicated the average drop from baseline in free plasma TRP, unless otherwise noted.

healthy males and females, before and after RTD. There were pronounced gender differences in 5-HT synthesis at baseline, prior to challenge, and this difference was compounded by RTD. At baseline, women had lower rates of synthesis at baseline than men (average synthesis rates in men were approximately 50% greater). Fur-

thermore, 5 hours post-RTD, 5-HT synthesis in men was reduced from baseline by a factor of ten by RTD, whereas in women RTD reduced 5-HT synthesis by a factor of forty.

This study indicated drastic reductions in synthesis in virtually all brain regions measured (frontal cortex,

parietal cortex, temporal cortex, occipital cortex, caudate, putamen, globus pallidus, thalamus, hypothalamus, amygdala, and hippocampus). PET measures of cerebral metabolism (e.g., Bremner et al. 1997, mentioned below) do not reveal anything specific about central 5-HT release, but the idea that RTD has CNS effects is supported by these studies.

Neuroendocrine Effects of RTD. Prolactin responses to d-fenfluramine were attenuated following RTD but not after sham depletion (Coccaro et al. 1998).

Polysomnographic Sleep Measures. A cluster of abnormalities in polysomnographically recorded EEG sleep — such as reduced latency to rapid eye movement (REM) sleep, and increased REM sleep duration, REM sleep percent of total sleep time, and REM density — are often associated with depressive illness (Benca et al. 1992). Administration of SSRI suppresses these REM sleep variables (e.g., Gillin et al. 1997) in depressed and non-depressed subjects.

In SSRI-treated fully remitted depressed men, we found that RTD induced depression-like increases in all REM sleep measures (Moore et al. 1998). In other words, RTD produced “depressed” sleep in remitted patients without clinically significant changes in scores on the Hamilton Depression Rating Scale (HDRS). Monoamine oxidase inhibitors (MAOIs) may suppress REM sleep even more effectively than SSRIs (Wyatt et al. 1969; Landolt et al. 1999). Preliminary data suggest that RTD disinhibits this REM sleep suppression and elicits large increases in REM sleep time without clinically significant depressive relapse in recently remitted phenelzine-treated depressed patients. For example, in one patient, REM time increased from zero minutes on phenelzine to 140 minutes on phenelzine and RTD, but not on phenelzine and sham challenge (Landolt et al. 2000). Polysomnographic sleep measures were also significantly affected by RTD in one report (Bhatti et al. 1998) but not in another (Voderholzer et al. 1998). Bhatti et al. (1998) reported that two strengths of RTD (100 and 25%) dose-dependently reduced REM latency compared to a baseline sleep night, and the 25% RTD significantly increased REM percentage. The RTD-induced disinhibition of REM sleep would be consistent with considerable data regarding serotonergic inhibitory control of REM sleep. In particular, it is consistent with animal data in which the withdrawal of 5-HT release quickly leads to onset of REM sleep (Portas and McCauley 1994; Portas et al. 1996).

RTD and Depressive Relapse: Does RTD Reverse the Beneficial Effect Treatment Preferentially with Different Types of Antidepressants?

Partial Remission, Antidepressant Treatment. The popularity of RTD resulted from several early studies in

which the antidepressant effects of SSRIs or MAOIs were temporarily reversed (Delgado et al. 1990, 1991, 1994). RTD, but not the TRP-containing sham challenge, induced a depressive relapse in 14 of 21 inpatients hospitalized for major depressive disorder who had responded well to antidepressant therapy (defined as a 50% improvement in HDRS, and symptomatic stability for two weeks) (Delgado et al. 1990) (see Table 3).

A “depressive relapse” was defined as an increase in pre-challenge HDRS score by at least 50% or a score >17. Mood gradually returned to pre-RTD levels with resumption of a normal diet. Case reports suggested that the individual’s symptom profile prior to treatment seemed to re-emerge following RTD. These findings with RTD are consistent with those using an earlier method of depleting serotonin, PCPA. In those studies, PCPA elicited depressive relapse in a similar fashion in MAOI-treated recently remitted patients (Shopsin et al. 1976). Its use was discontinued because of adverse side effects.

It bears mentioning that “depressive relapse” has been defined differently. Here, for the purpose of comparison, a single set of relapse criteria was employed. The relapse criteria defined by Delgado et al. (1990) is the most restrictive: an increase in pre-challenge HDRS score by at least 50% and >17. Lesser changes in depressive symptoms as measured by HDRS are meaningful, but the relationship to full clinical relapse is unclear.

In patients treated with antidepressants such as MAOIs or SSRIs that primarily enhance 5-HTergic neurotransmission, RTD led to an 80% rate of relapse (16 of 22 patients) (Delgado et al. 1991). Only 18% (two of 13) of desipramine- or bupropion-treated (reuptake inhibitors of norepinephrine or dopamine, respectively) patients relapsed with RTD (Delgado et al. 1991, 1993, 1994). A subsequent study directly compared RTD responses in patients recently treated with fluoxetine vs. desipramine. Relapse occurred in eight of 15 fluoxetine-treated patients, compared with only one of 15 on desipramine (Delgado et al. 1999).

Full Remission. Whether drug-free or still on AD treatment, fully remitted patients who have been euthymic for at least two months relapse very rarely with RTD. Moreno et al. (1999) compared mood responses to RTD in twelve fully remitted drug-free patients with twelve subjects with no history of depression. Only the patients experienced depressive changes measured by HDRS. Again, full clinical relapse was rare, and mood alterations in general were highly variable in magnitude and time course. In a study by Smith et al. (1997), five of 15 euthymic women with a history of depression were reported to relapse, but possibly only two of these met criteria for full clinical relapse (as defined by Delgado et al. (1990)). Even lower relapse rates were found in other studies; Leyton et al. (1997) found no mood changes following RTD in 14 drug-free

Table 3. Effects of RTD in Medicated Patients

N	Diagnosis	Ref.	Medication Status	Remission Status	% TRP Decline	Relapse Rate	Comments
21# (13F, 8M)	Major depression	Delgado et al. 1990	4 weeks of antidepressant treatment: 9 on SSRI (fluvoxamine), 7 on TCA (desipramine, imipramine), 5 on MAOI (phenelzine, tranylcypromine)	Partial (2 weeks)	87%	14 of 21 (SSRI: 7 of 9 TCA: 2 of 7 MAOI: 5 of 5)	Relapse defined as an increase in HDRS score by 50% and ≥ 17 . Relapse experience closely resembles the patient's index episode.
115#: (7F, 7M)	Major depression 69 drug-free and symptomatic, 46 treated and partially remitted	Delgado et al. 1991	69 untreated: no medication 46 treated: 13 with NERI/DARI 22 with SSRI/MAOI	Untreated patients were not remitted; Treated patients—partially remitted (about 2 weeks)	81%	Untreated pts: 0 of 69 Treated pts: 24 of 46	In the untreated, fully symptomatic patients, mood does not worsen on the day of testing. On Day 2, about 37% have 10-pt improvement on HDRS score, and about 23% have 10-pt worsening on HDRS. In the treated patients on serotonin-enhancing drugs, 16 of 22 relapse (6 of 6 on MAOI, 10 of 18 on SSRI). 2 of 13 relapsed that had been on imipramine, desipramine, or bupropion. 1 of 1 treated with amphetamine relapsed.
15# (7F, 7M)	Major depression	Delgado et al. 1994	4 weeks of antidepressant treatment	Partial (2 weeks)	n.r.	9 of 15	Subjects had been part of a larger group (n=43) that were initially challenged with RTD prior to beginning antidepressant treatment. Mood did not worsen on RTD day in any of the 43. With the 2 nd RTD trial, after patients had partially remitted, 9 of 15 experienced a dramatic worsening of mood and relapse of depressive symptoms. Breakdown of type of antidepressant treatment of the 15 remitted patients was not reported.
30 (15F, 15M)	Major depressive episode	Delgado et al. 1999	15 patients (8F, 7M) were recently treated with SSRI (fluoxetine); 15 patients (6F, 9M) were treated with TCA (desipramine)	Partial (2 weeks)	77%	8 of 15 on SSRI 1 of 15 on TCA	Only about 1/3 rd of subjects received a TRP-containing placebo control; the others received a 25g RTD as a sham depletion. The 25g RTD led to a slight decrease in plasma TRP. This study controlled for previous factors (melancholic symptoms, treatment refractoriness) that had been associated with RTD-induced relapse.
21 (10F, 11M)	Major depression	Bremner et al. 1997	On SSRI for 1 to 335 weeks	Some partial, some fully remitted	75%	7 of 21	Modified criteria for relapse: increase in HDRS of 50% and 9 points. PET scan measurements of metabolic activity conducted at baseline and after each challenge. Relapses show decreased metabolic activity in thalamus, medial frontal gyrus and orbitofrontal cortex. One patient relapsed with sham depletion.

continued

Table 3. (continued)

N	Diagnosis	Ref.	Medication Status	Remission Status	% TRP Decline	Relapse Rate	Comments
20 (9F, 11M)	Major depressive disorder in clinical remission	Aberg-Wistedt et al. 1998	Responders to SSRI citalopram	Partial: at least one month (some may be longer)	52%	Not defined	Used a modified RTD mixture: 43g of amino acids. Did not use the HDRS, but the Montgomery-Asberg rating scale for depression. Remission and relapse were not defined. Pre-RTD cortisol levels were higher in patients who experienced a response to RTD.
10 M	Major depressive episode in full clinical remission	Moore et al. 1998	Serotonin reuptake inhibitors (fluoxetine, paroxetine, sertraline)	Fully remitted	100g: 52% 25g: 6%	0 of 10	Administered 25g and 100g RTD challenges at 3pm and then recorded EEG sleep all night. There were no clinically significant changes mood changes. There were, however, significant alterations in all REM sleep measures (increased REM %, increased REM minutes, increased REM density, reduced latency to REM sleep), indicating the RTD had disinhibited REM sleep.
15 (9F, 6M)	Obsessive compulsive disorder; (10 with history of major depression)	Barr et al. 1994	All on SSRI at test time for between 5 to 104 weeks; two were also on other sedatives	Successfully treated with SSRI at test time	84%	OCD: 0 of 15 Depression: 5 of 15	Used the Yale Brown Obsessive Compulsive scale to measure obsessive compulsive symptom changes; there were no clinician- or patient-related worsening of obsessional symptoms with RTD. However, there were increases in depressive symptoms measured by HDRS: five patients met Delgado et al. (1990) criteria for relapse.
16 (10F, 6M)	Schizophrenia or schizoaffective disorder	Sharma et al. 1997	2 were drug free; others were maintained at dose of non 5-HT neuroleptic at test time	Positive & negative schizophrenic symptoms present at test time	57%/(total)	Not applicable	An improvement in schizophrenic symptoms following RTD was expected, but did not obtain. HDRS was not used; used the Brief Psychiatric Rating Scale and the Scale for Assessment of Negative Symptoms. There was no change in positive symptoms, there was some deterioration in negative symptoms. Sham challenge contents had been modified slightly due to unavailability of TRP except in a commercially prepared multi amino acid preparation.

Abbreviations: SSRI = selective serotonin reuptake inhibitor; TCA = tricyclic antidepressant; MAOI = monoamine oxidase inhibitor; HDRS = Hamilton depression rating scale; REM sleep = rapid eye movement sleep; OCD = obsessive compulsive disorder.
 # includes overlap of patients reported other studies by same authors.
 n.r. = not reported.
 % TRP decline indicates the average drop from baseline in free plasma TRP, unless otherwise noted.

recovered patients, and Moore et al. (1998) found no relapse in 10 fully-remitted SSRI-treated patients, even though RTD significantly lowered plasma TRP concentrations and induced depression-like sleep alterations (shortened REM latency, and increased REM percent and REM density).

With the exception of Bremner et al. (1997), whose subjects had been on SSRIs for two to 337 weeks and most likely included some recently remitted patients, there has been little additional data in recently remitted pharmacologically treated patients.

Momentary 5-HT availability may be critical for maintaining mood only during certain critical time periods in antidepressant-induced recovery (Blier and de Montigny 1994; Artigas et al. 1996), but not at other time points. We speculate that this temporary dependence on 5-HT in early stages of remission may account for some of the variability in mood effects of RTD.

The current evidence suggests that the RTD-induced relapse of depressive symptoms is more likely earlier in remission rather than later, and when treated with SSRIs or MAOIs compared with catecholamine-enhancing drugs.

Obsessive-Compulsive Disorder and Panic Disorder, Symptomatic and Untreated. Obsessive-compulsive symptoms in SSRI-treated or drug-free obsessive compulsive disorder patients did not worsen following RTD, in either subjective or clinician rated assessment scales (Barr et al. 1994) (see also Table 4 in unmedicated patients) (Smeraldi et al. 1996). Depressive relapse was seen in five of 15 patients with obsessive compulsive disorder, some of whom also had some depressive symptoms prior to RTD challenge (Barr et al. 1994).

Other Psychiatric Diagnoses

Late Luteal Phase Dysphoric Disorder (LLPDD), or Premenstrual Syndrome. Female patients with monthly depression, or late luteal phase dysphoric disorder (LLPDD), did not experience changes in HDRS after RTD (Menkes et al. 1994); however, RTD did elicit significant increases in subjectively-rated LLPDD-related symptoms compared with sham depletion. Each of these patients underwent two challenges during each of two phases of the menstrual cycle, the follicular phase and the luteal phase. During the follicular phase, RTD induced an LLPDD-like state in a subgroup of the women, and during the luteal phase, RTD significantly worsened ratings of irritability. A group of normal comparison subjects would have helped to illuminate whether women in general are more vulnerable to mood effects with RTD during different phases, and this topic will be returned to below. Menstrual physiology may significantly impact RTD results in only a subgroup of women, yet until these factors are better un-

derstood, it is probably prudent to control for cycle phase.

Autism. Eleven of 17 autistic adults showed a significant worsening of symptoms following RTD with both clinically based and subjectively rated symptom assessments (McDougle et al. 1996). RTD but not sham depletion led to increases in autistic behaviors (such as whirling, flapping, pacing, banging and hitting self, rocking and toe walking, etc.) and alterations in emotional states.

Cocaine Craving and Reward. In cocaine addicts, RTD affected the subjective craving for cocaine as well as its rewarding qualities. When patients were exposed to cocaine-related paraphernalia (specifically intended to enhance craving), RTD diminished the subjective feelings of craving (Satel et al. 1995). In another group of addicts, RTD diminished the "high" feeling after intranasal cocaine when compared with sham challenge (Aronson et al. 1995). Since serotonergic raphe neurons are known to modulate mesolimbic dopaminergic reward pathways believed to mediate the rewarding properties of all known drugs of abuse (Baumgarten and Grozdanovic 1994), these findings imply that RTD directly reduces the pleasurable subjective states related to drug craving and drug reward.

Bulimia. In bulimic women, whether recently symptomatic or abstinent for six months, RTD has inconsistent mood lowering effects (Weltzin et al. 1994, 1995; Oldman et al. 1995). However, RTD-induced effects on food choices in a test meal following RTD, as well as increased irritability, and changes in eating cognitions and self-perceptions have been observed. By contrast, changes in healthy volunteers' food choices have been subtle (Young et al. 1988; Oldman et al. 1994). Smith et al. (1999) found that drug-free women with a history of bulimia and major depression had a modest lowering of mood (mean change in HDRS ~8 points), and increases in body-image and eating-control related fears and cognitions. Normal comparison subjects did not show these changes.

Tourette's Syndrome. Unmedicated Tourette's syndrome patients did not show worsening of clinician-rated tic, obsessive/compulsive, or mood symptoms with RTD (Rasmussen et al. 1997).

Intermittent Explosive Disorder. Other negative findings include clinician-rated aggressive behavior in patients hospitalized with intermittent explosive disorder (Salomon et al. 1994). By comparison, effects of RTD on measures of aggression in healthy subjects are also somewhat mixed. In some but not all studies, significant increases in RTD-increased aggressive responding in experimentally-controlled situations have been re-

Table 4. Effects of RTD in Unmedicated Psychiatric Patients

# PTS	Diagnosis	Ref.	Symptomatic At Test Time	Clinician Rated Measures	Self-Rated Measures	% TRP Decline	Rate of Symptom Change/Worsening	Comments
43# (22F, 21M)	Major depression	Delgado et al. 1994	Fully symptomatic	HDRS	None	83%	None of 43	Mood did not worsen in any of the 43 subjects on day of RTD challenge. However, Day 2 responses were variable: some improved, some worsened.
16F ^a	Late luteal phase dysphoric disorder (4 were on SSRI)	Menkes et al. 1994	Each subject tested once during follicular phase and once during luteal phase	(none)	Self-report survey of premenstrual symptoms	78%	14 of 16 worsened when tested during luteal phase	RTD induced certain premenstrual symptoms to occur during follicular phase. When tested during follicular phase, RTD exacerbated symptoms less dramatically than luteal phase testing.
17 (2F, 15M)	Autistic disorder	McDougle et al. 1996	Moderately severe symptoms at test time	Behaviors (whirling, flapping, pacing, hitting self, toe walking, rocking); global severity scale, Clinician-rated VAS scales	None	69%	11 of 17 worsened	In addition, VAS scales of calmness, happiness were lower; anxiety was higher with RTD. No observable changes in other behaviors. Patients who worsened with RTD had higher scores on baseline tests.
25M	Cocaine dependence	Satel et al. 1995	In rehab at test time	None	Beck Depression Inventory, VAS ratings of craving post exposure to cocaine materials, of anxiety and mood	40%	6 of 10 reported LESS craving after RTD	Habituation (second exposure in counterbalanced crossover design) also played role in lessening of craving.
12 2F, 10M	Cocaine dependence	Aronson et al. 1995	In rehab at test time	None	VAS responses following intranasal cocaine	78%	9 of 12 reported feeling less "high" after RTD than after sham challenge	An order effect emerged in the analysis, affecting interpretation of results.
13 F ^a (plus 9F controls)	Bulimia	Weltzin et al. 1994	Inpatients in eating disorders program	6-pt ratings on psychological scales of: anxiety, depression, body image, over-reactivity, indecision, fatigue, guilt, hostility, elation, obsessive thinking, physical activity, desire to binge or purge	None	64%	Individual effects not reported. Bulimics showed increased fatigue after RTD. Controls were rated as more depressed and overreactive after RTD	Bulimics had increased fatigue on RTD, more fear of fat. Also showed that sham challenge has prominent effects on Trp-LNAA ratio. Diminishes Trp to 45%, compared with 10% on RTD.

continued

Table 4. (continued)

# PTS	Diagnosis	Ref.	Symptomatic At Test Time	Clinician Rated Measures	Self-Rated Measures	% TRP Decline	Rate of Symptom Change/Worsening	Comments
10F ^a (plus 10F controls)	Bulimia and major depressive episode	Weltzin et al. 1995	Inpatients in eating disorders unit, also symptomatic for depression	Same as Weltzin et al. 1994	None	80%	6 of 10 bulimics ate more in a test meal following RTD, than in a meal following sham challenge	No increase in depressed mood in depressed women; no change in healthy women. Bulimics as a group were more irritable after RTD.
8F (plus 12F controls)	Abstinent bulimia, (6 with history of major depression)	Oldman et al. 1995	Abstinent at least 6 months, and up to 27 years	Food choices in a "buffet lunch" test meal	40-item Eating Attitudes test, Beck Depression Inventory, POMS, VAS	80% (pts)	No consistent lowering of mood. No effect on food choices	Used a 50g RTD. RTD did not produce changes in self-reported depression ratings, nor in eating attitudes, although bulimics ate less food following RTD. Bulimics also had lower total plasma TRP (but not lower free TRP levels) than controls at baseline.
12F, (plus 12F controls)	History of bulimia and major depression	Smith et al. 1999	Clinically recovered for at least 6 months	HDRS 17-item	VAS ratings of feeling fat, or uncontrolled urge to eat	n.r.	Group increase in depression ratings (specifics not reported)	Used an 86g RTD. 6 of 10 bulimics (but none of the controls) reported at least a mild increase in eating disorder cognitions. Bulimics with the highest HDRS scores were bulimics with a history of major depression.
12 (8F, 4M)	Obsessive compulsive disorder	Smeraldi et al. 1996	Symptomatic—seeking treatment in inpatient psychiatric unit	Y-BOCS, HDRS	VAS	73%	No exacerbation of symptoms	Used 38% strength RTD challenge.
6 (2F, 4M)	Tourette's syndrome with either Obsessive compulsive disorder or obsessive-compulsive features	Rasmussen et al. 1997	Variable	Clinician rated tics, Yale Brown Obsessive Compulsive Scale, HDRS	Subjective urge to tic, Buss-Durkee Hostility Inventory, POMS	87%	No effects on clinician-rated measures, no increases of tics or other TS symptoms	Increased subjective urge to tic and increased POMS anxiety.
8 (4F, 4M)	Panic disorder	Goddard et al. 1995	Symptomatic—seeking treatment in inpatient psychiatric unit	(none)	VAS, and panic attack questionnaire		0 of 8 panic attacks	1 of 8 had panic attack with sham depletion.

continued

Table 4. (continued)

# PTS	Diagnosis	Ref.	Symptomatic At Test Time	Clinician Rated Measures	Self-Rated Measures	% TRP Decline	Rate of Symptom Change/Worsening	Comments
5 (2F, 3M)	Panic disorder	Kent et al. 1996	Symptomatic—seeking treatment in inpatient psychiatric unit	Observer ratings of panic; increased ventilation	CO ₂ challenge and hyperventilation: Acute panic inventory, 10-pt scales of anxiety and apprehension; measure of breathlessness	80–86%	No panic attacks elicited in either panic disorder patients nor normal control subjects	RTD did not significantly increase anxiety. No change in respiratory variables in controls following RTD.
14 (1F, 13M)	Aggressive inpatients with intermittent explosive disorder	Salomon et al. 1994	Variable	Buss-Durkee Hostility Inventory, Overt Aggression Scale	None	82%	No changes in hostility in hospital environment	RTD induced an unexpected decrease in irritability.
18M (plus 20 controls)	Aggressive adolescent males	LeMarquand et al. 1998	"Aggressives" had been previously characterized and were stable at test time	"go/no-go" learning task	None	81%	No effects of RTD on the learning task in either group	There were group differences between the groups in impulsivity and executive function: stable aggressives made more commission errors, even after controlling for IQ, years of education and family revenue.

Abbreviations: HDRS = Hamilton depression rating scale; SSRI = selective serotonin reuptake inhibitor; VAS = visual analog scale; TRP = tryptophan; LNAA = large neutral amino acid; POMS = profile of mood states; Y-BOCS = Yale-Brown obsession-compulsion scale.
 * indicates a study using female subjects in which phase of menstrual cycle was controlled.
 # includes overlap of patients reported other studies by same authors.
 n.r. = not reported.
 % TRP decline indicated the average drop from baseline in free plasma TRP, unless otherwise noted.

ported (Moeller et al. 1996; Bjork et al. 1999; LeMarquand et al. 1998; Cleare and Bond 1995).

Schizophrenia. Schizophrenic patients with positive and negative symptoms had been expected to improve with RTD, but did not. In fact, negative symptoms worsened slightly, thus not supporting a clear role for serotonin in these clinical symptoms (Sharma et al. 1997).

RTD also failed to develop panic attacks in medication-free, symptomatic panic disorder patients (see Table 4) (Goddard et al. 1995; Kent et al. 1996). These two studies do not necessarily negate serotonergic involvement in panic, anxiety and related symptoms. Other reports indicate significant respiratory responses to RTD in panic disorder patients but not normal comparison subjects (Kent et al. 1996). Increases in anxiety responses to CO₂ challenge were seen in healthy subjects (Goddard et al. 1995; Klaassen et al. 1998). Enhancements in subjects' ratings of nervousness in response to i.v. yohimbine (an α_2 norepinephrine antagonist that often leads to a worsening of anxiety symptoms) were seen with RTD compared to control testing (Goddard et al. 1995).

What Effect Does RTD Have on Symptomatic Unmedicated Patients?

In 49 untreated (drug-free) fully symptomatic depressed patients, RTD induced little mood change (Delgado et al. 1993). After treatment with various types of AD (TCA, SSRI, MAOI), 15 of these patients were re-challenged with RTD after four weeks' remission. Nine experienced a depressive relapse upon the repeat-RTD day. Whether type of AD was associated with relapse here was not reported.

Similar to the RTD approach, the relapse-inducing effects of depleting catecholamines (with α -methyl-*para*-tyrosine, AMPT, a blocker of catecholamine synthesis) have also been investigated (Miller et al. 1996; Heninger et al. 1996). AMPT-induced changes mimic those with RTD in terms of effects in recently remitted patients with specific pharmacotherapies. Specifically, AMPT induced relapse in all recently remitted patients on catecholamine-enhancing drugs (five of five on desipramine, a norepinephrine reuptake inhibitor; or mazindol, a dopamine reuptake inhibitor), whereas only one of nine SSRI-treated patients relapsed with AMPT. Similar to RTD, AMPT had no effect on HDRS scores when given to acutely ill drug free depressed patients, despite changes in plasma levels of catecholamine metabolites. Interestingly, though tyrosine is the substrate for catecholamine synthesis, in a pathway analogous to that for TRP and 5-HT, acutely depleting the amino acid tyrosine is not typically the approach used to deplete catecholamines. This is believed to be so because of two critical differences between tyrosine and

TRP. For one, tyrosine availability is not the rate-limiting step in synthesis. Second, tyrosine is not an essential amino acid, since the liver can synthesize it from another amino acid, phenylalanine (Fernstrom and Wurtman 1974) (see Table 1).

RTD and Physiologic Variables Associated with Depression

PET measures of cerebral glucose metabolism were taken in SSRI-treated euthymic depressed patients following RTD (Bremner et al. 1997). In patients who relapsed, post-RTD PET scans indicated decreases in brain metabolism in dorsolateral prefrontal cortex, thalamus, and orbitofrontal cortex, compared to pre-RTD baseline metabolic measurements. Patients not relapsing with RTD did not show these changes. In addition to post-RTD changes, patients who relapsed may have been different from non-relapsers in baseline metabolic measurements of prefrontal and limbic regions.

Endocrine responses to serotonergic drug challenge also differentiate depressives from healthy controls. Pituitary release of several hormones is triggered by serotonergic activation of hypothalamic hormone-releasing signals. In particular, serotonergic drugs appear to enhance release into plasma of prolactin, growth hormone, and ACTH in healthy volunteers. These measures are consistently decreased in depressed patients in response to general serotonergic challenge with L-TRP or the serotonin releaser, fenfluramine (Cowen 1993). These lowered neuroendocrine responses to serotonergic challenges in depressed patients are thought to reflect pathologically diminished levels of serotonin release in depression. If so, RTD should lower these already abnormally low responses to 5-HT drug challenge. However, if RTD-associated attenuation of 5-HT release induces alterations in the functional sensitivity of 5-HT receptors, neuroendocrine responses to 5-HT challenge will reflect this.

Price and colleagues have demonstrated the latter. In drug-free depressed patients, cortisol responses to intravenous infusions of TRP were significantly greater during RTD than during sham depletion (Price et al. 1998). Similarly, in drug-free depressed patients, i.v. infusion of mCPP (a 5-HT_{2A/2C} receptor agonist) affected serum cortisol greater after RTD than did sham depletion. These enhanced neuroendocrine responses are suggestive of upregulated 5-HT receptor function, presumably a response to lowered 5-HT release (Price et al. 1997).

Does RTD Reverse Other Beneficial Treatments in Mood Disorders?

In recently remitted patients treated with non-pharmacological interventions hypothesized to have a 5-HT-enhancing basis of action, RTD induces full clinical re-

lapse only rarely (see Table 5). Variable effects on mood have been reported in depressed patients treated with electroconvulsive therapy (Cassidy et al. 1997), in depressed and bipolar patients euthymic following one night of total sleep deprivation (Neumeister et al. 1998a), or in seasonal affective disorder patients treated with bright light therapy (Lam et al. 1996; Neumeister et al. 1997, 1998b; Lenzinger et al. 1999). Cases of RTD-induced full clinical relapse (meeting Delgado et al. (1990) criteria) are rare. In some cases, RTD-induced mood effects occurs outside the time window of plasma TRP depletion, and after plasma TRP has repleted to baseline levels. There have been no RTD investigations in recently remitted patients treated with cognitive behavior therapy or interpersonal psychotherapy alone.

Manic Symptoms. In terms of manic symptoms in patients with bipolar disorder, RTD again elicits mixed responses. Fully remitted patients treated with lithium for a minimum of one year show no significant changes in either depressive mood or manic symptoms (Benkelfat et al. 1995). There are two reports in recently remitted lithium-treated mania. Capiello et al. (1997) found exacerbated mania in two of seven patients, though Cassidy et al. (1997) reported no change in four patients. Taken together, this suggests that lithium may exert its beneficial effects on the serotonin system in only a subset of patients.

RTD's Effects on Unaffected Subjects at Risk for Depressive Illness

Mood Effects in Healthy Volunteers Predisposed to Depression. Genetic, familial, or gender-related vulnerability to depressive disorders in never-depressed subjects has provided intriguing, yet mixed, results (see Table 6). Scores on the depression subscale of the self-rated Profile of Mood States (POMS) were significantly more affected after RTD in males who had a strong family history of depression than in never-depressed males with no family history (Benkelfat et al. 1994). Healthy, non-depressed women have shown RTD-induced worsening in POMS subscales (Ellenbogen et al. 1996). In one report, healthy non-depressed women with a family history of depression were more likely to show subtle mood and gastrointestinal changes than those with no family history (Klaassen et al. 1999b), but another investigation in healthy non-depressed family-history-positive women showed no change in mood ratings (Ellenbogen et al. 1999).

RTD, Mood, and Physiologic Variables in Healthy Subjects

Mood Changes. In healthy subjects, clinically significant RTD-induced changes of mood are rare and gener-

ally mild (see Table 7). No study reports the induction of a depressive state measured by HDRS in well-screened healthy subjects. Mood changes are typically subtle and measurable only using subjective rating scales, and even these have been inconsistently reported. Mood-lowering effects on the depression subscale of the Multiple Affective Adjective Checklist (MAACL) have been reported in healthy young men (Young et al. 1985). However, a lack of significant mood consequences using these and other subjective mood assessments has also been reported by Danjou et al. (1990), Abbott et al. (1992), and Oldman et al. (1994). In addition, healthy subjects who were administered SSRIs for six weeks and then challenged with RTD did not produce any measurable changes in mood (Barr et al. 1997).

Higher Cognitive Function, Neuroendocrine Measures, Immune Responses, and Pain Perception. There are also reports of RTD-induced alterations in attention (Coull et al. 1995), and RTD-induced impairments in performance on certain types of learning and memory (Park et al. 1994; Riedel et al. 1999). A variety of immune responses were not substantially altered by RTD (Ravindran et al. 1999). Pain threshold and pain tolerance were not affected by RTD nor sham challenge, but RTD reduced the analgesic effect of morphine (Abbott et al. 1985), suggesting serotonin's influence on pain pathways.

Are There Non-Serotonergic Explanations for the Effects of RTD?

The tryptophan-free challenge contains other amino acids, some of which possess neuroactive or neurotransmitter-like properties (such as glycine (Gly), and Gly receptors can also be activated by alanine, serine, and proline). Other amino acids such as tyrosine are precursors for other neurotransmitters (dopamine and norepinephrine). Given that these amino acids are present in the same amounts in TRP-free and TRP-containing preparations, and that sham depletion techniques have measurable effects only rarely, the discrepancy between the effects seen with TRP-free and TRP-containing challenges therefore cannot be accounted for by simply having taken in a huge bolus of amino acids. In support of this, Klaassen et al. (1999a) depleted healthy subjects either of TRP or lysine, using a similar preparation. Unlike RTD, rapid lysine depletion provoked no measurable mood or memory effects. This is taken as evidence that RTD's effects are indeed due to the specific depletion of tryptophan.

If it can be assumed that RTD reduces 5-HT release, other neurotransmitter systems may be affected. In this way, the RTD paradigm does not necessarily indict the 5-HT system as neuropathological,

Table 5. Effects of RTD on Mood and Mania with Non-Pharmacologic Types of Treatment

N	Scid Diagnosis	Ref.	Type of Treatment	Remission Status	% TRP Decline	Relapse Rate	Comments
5 (3F, 2M)	Major depressive disorder	Cassidy et al. 1997	Electroshock therapy	Partial (1–4 days)	85%	0 of 5	No significant changes with MADRS scale scores.
11 (8F, 3M)	Major depression, bipolar I, II	Neumeister et al. 1998 ^a	1 night of total sleep deprivation (TSD); otherwise drug-free	Partial (hours)	85%	0 of 11	None of the patients responding to TSD relapsed on RTD day. Interesting mix of day 2 effects. Unlike most other studies, this was not a counterbalanced crossover design. 30 subjects total in the study, 22 of which responded to TSD. 11 of these 22 received RTD challenge, the other 11 received sham challenge.
10M	Bipolar I	Benkelfat et al. 1995	Li++-treated for at least one year	Full	85%	0 of 10	No significant effects on depressive mood, nor of mania.
7 (4F, 3M)	Bipolar I, manic episode	Cappiello et al. 1997	Li++-treated	Partial (recently manic); 2 weeks to one month	n.r.	2 of 7 (manic symptoms)	1 patient had exacerbated mania with sham depletion.
4 (2F, 2M)	Bipolar, recently manic	Cassidy et al. 1997	Li++-treated	Partial: one month	75%	0 of 4	No change in either depression or mania ratings.
12 ^b (10F, 2M)	Seasonal affective disorder	Neumeister et al. 1997	Bright light therapy; drug-free	Partial: 16–38 days	80%	6 of 12	Note the different criteria for depressive relapse: an increase in HDRS of 50% and ≥ 12 . Scores on day after test day were similar to scores prior to light treatment. Atypical symptoms of SAD were more prominently affected by RTD than other depressive symptoms.
18 ^c (16F, 2M)	Seasonal affective disorder	Lenzinger et al. 1999	Bright light therapy	Partial: stable for 2 weeks	80%	6 of 10	Note the different criteria for SAD relapse: HDRS score of ≥ 12 . Some overlap of patients reported in Neumeister et al. 1997.
10 (7F, 3M)	Seasonal affective disorder	Lam et al. 1996	Bright light therapy	Partial: 3–7 weeks	80%	6 of 10	Note the different criteria for SAD relapse: HDRS score of ≥ 12 . 2 of 10 had increases in anxiety scores. Mood changes occurred on testing day, unlike Neumeister et al. 1997.
11 (9F, 2M)	Seasonal affective disorder, winter pattern	Neumeister et al. 1998	Drug-free and spontaneously remitted (tested during summer)	Full	85%	8 of 11	Note the criteria for SAD relapse: increase in SIGH-SAD (modification of HDRS) by 50% of baseline.

Abbreviations: MADRS = Montgomery-Asberg depression rating scale; HDRS = Hamilton depression rating scale; SAD = seasonal affective disorder.

^a includes overlap of patients reported other studies by same authors.

n.r. = not reported.

% TRP decline: average drop from baseline in free plasma TRP, unless otherwise noted.

Table 6. Effects of RTD in Unaffected (Non Depressed) Subjects at Risk for Mood Disorders

Ref.	N	Mood Measure	% TRP Decline	Effects and Comments
Benkelfat et al. 1994	39 M, with or without a family history of depression	POMS, HDRS, Beck depression inventory, VAS.	89%	6 of 20 family history positive subjects showed a 10-pt change in POMS—depression subscale; none of 19 family history negative subjects had such a response. No indication of depressive mood in other measures.
Ellenbogen et al. 1996	20 F ^a , without family history of depression	POMS, VAS: females screened to be family history negative	80%	Used an 86g challenge. Four POMS subscales (Depression, Tired, Unsure, Confused) indicated a lowering of mood. These mood effects were lost on re-test one month later.
Ellenbogen et al. 1999	14 F ^a , with family history of depression	POMS, VAS: FH + for major affective disorder (three family members in two generations)	85%	Family history was defined by presence of at least three affected family members in two generations. Used an 86g challenge. Counter to prior report, there were no significant effects on mood measures.
Moreno et al. 1999	12 drug free patients with history of depression (8F, 4M)	HDRS	85%	Two patients with history of depression met relapse criteria of Delgado et al. (1990). No control subjects became depressed.
Leyton et al. 1997	14 ^a drug free patients with history of depression (8F, 6M)	HDRS	88%	No patients experienced a depressive relapse. In fact, there were no measurable changes in mood.
Smith et al. 1997	15F with history of multiple episode history of depression	HDRS	75%	Five of 15 were reported to relapse using different criteria. Fewer than 5 (estimate: 2) met Delgado et al. (1990) criteria for relapse.

Abbreviations: POMS = profile of mood states; HDRS = Hamilton depression rating scale; VAS = visual analog scale.

^a indicates a study including female subjects in which menstrual cycle phase was controlled for.

\$ indicates a study including female subjects in which menstrual cycle is addressed but not controlled for.

% TRP decline: average drop from baseline in free plasma TRP, unless otherwise n.

it simply uncovers other systems being modulated, modified, or regulated by 5-HT. As stated previously, the projection sites of 5-HT neurons extend over much of the CNS, and the scope of 5-HT's influence may be vast. Sudden withdrawal of 5-HT's presumed modulatory presence might result in counterregulatory changes in other systems.

It is also unknown what a sudden drop in TRP levels does to enzymatic activity of TRP hydroxylase (TRP-H). There is evidence that dramatic diminution of brain TRP elicits increases in TRP-H activity (Neckers et al. 1977). It is unknown to what extent putative increases in TRP-H activity affect total 5-HT synthesis and release, but a compensatory mechanism such as this could theoretically offset any decrement in 5-HT production.

It is unknown how much of the body's total TRP is used for 5-HT metabolism (Kuhn et al. 1986). It is also possible that depleting TRP via RTD affects the synthesis of other TRP-containing compounds, and not (only) the synthesis of 5-HT. Since TRP is an amino acid and a constituent of many proteins — including various neurohormones and neuropeptides — depleting TRP might affect de novo synthesis of these products. Theoretically,

a sudden drop in level of neuroactive substances like these might be responsible for the effects of RTD.

For example, with regard to the REM sleep disinhibiting effects of RTD: the so-called delta-sleep inducing peptide (DSIP) contains TRP at its N-terminus (TRP-Ala-Gly-Gly-Asp-Ala-Ser-Gly-Glu). DSIP has been postulated as a substance that accumulates in the brain during wakefulness, and is discharged or dissipated during delta non-REM sleep. If this is so, then RTD-induced decline in DSIP production, rather than diminished 5-HT synthesis, might account for the reduced REM latencies associated with RTD. By contrast, other substances that might also have accounted for the RTD-induced REM-hastening effect — such as vasoactive intestinal peptide, which decelerates the non-REM-REM cycle (Murck et al. 1996) — do not contain TRP.

TRP is a constituent of somatostatin, for example. Lowered CSF levels of somatostatin, lowered blood levels of somatostatin-like immunoreactivity, or abnormalities in somatostatin gene expression have been associated with depressive mood in patients (Rubinow et al. 1983; Dinan 1998; Markou et al. 1998) or in animal models of depression (Gomez et al. 1999;

Table 7. Other Miscellaneous Effects of RTD

Behavior of Interest	Ref.	N	Mood Measure(s)	Other Measure(s)	% TRP Decline	Effects and Comments
MOOD	Young et al. 1985	36 M	Multiple Affective Adjective Checklist; and "distractibility" on a proofreading task with dysphoric stimuli	(None)	60%	Scores on both tests indicated a mild depressive state following RTD.
MOOD	Danjou et al. 1990	18 M	VAS, dysphoric distractor	(None)	77%	No differences in mood were associated with RTD, but group differences at baseline complicate interpretation of results.
MOOD	Barr et al. 1997	6 (2F, 4M)	VAS, HDRS, POMS. On fluoxetine for 6 weeks	(None)	88%	No change in mood on any scale following RTD.
MOOD	Smith et al. 1987	40 M	Multiple Affective Adjective Checklist, Depression Adjective Checklist.	(None)	76–83%	MAACL scores on depression subscale (but not anxiety nor hostility subscales) showed mild elevation after RTD.
ANXIETY & PANIC	Goddard et al. 1995	11 (1F, 10M)	VAS for anxiety, nervousness, plus Panic Attack Questionnaire	Responses to yohimbine, an adrenergic antagonist, that typically produces anxiety	N.R.	RTD plus yohimbine produced more "anxious" and "nervous" ratings than either preparation did alone. RTD plus placebo had no measurable effects on mood.
ANXIETY & PANIC	Koszycki et al. 1996	40 M	(None)	Behavioral & cardiovascular (panicogenic) effects of cholecystokinin agonist, CCK-4.	92%	CCK-4 did elicit panic attacks in 55% of RTD subjects and in 65% of sham challenge subjects. RTD, however, did not affect measures of panic.
ANXIETY & PANIC	Klaassen et al. 1999a	15 M	Anxiety and panic symptoms POMS-tension, Spielberger State Anxiety Inventory (STAI)	Neurovegetative panic symptoms	N.R.	RTD associated with increases in STAI, in POMS-tension, and in panic symptoms, but no effects on VAS-anxiety.
AGGRESSION	Moeller et al. 1996	10 M	(None)	Aggressive responding with point subtraction aggression paradigm	65%	RTD induced increased aggressive responding compared with the "control challenge". This was a 25% strength challenge, intended as placebo but had effects intermediate.
AGGRESSION	Cleare & Bond. 1995	48M	VAS—hostility, Spielberger anxiety and anger inventories, aggression rating scale, bodily symptom scale	Used a competitive reaction time task	80%	Half the subjects scored high on trait aggression; the other half scored low. In the high-aggression group, RTD was associated with increased subjective ratings of aggression as well as increased aggressive-like scores on the RT task. RTD induced no changes in the low-aggression group.
AGGRESSION	Bjork et al. 1999	8M	(None)	Aggression with the Point Subtraction Aggression Paradigm	N.R.	RTD induced increased aggressive responding but not increased money-earning responding. Extra condition of fasting (without challenge ingestion) did not significantly affect responding.

continued

Table 7. (continued)

Behavior of Interest	Ref.	N	Mood Measure(s)	Other Measure(s)	% TRP Decline	Effects and Comments
LEARNING AND MEMORY	Park et al. 1994	12 M	VAS	Neuropsychological battery including memory tests, visual discrimination, paired associate learning, rapid visual information processing	~ 20% (total TRP)	After RTD, subjects needed more trials to learn certain tasks, and made more errors in specific learning tasks (paired associates), among the many neuropsych tests performed
LEARNING AND MEMORY	Coull et al. 1995	56 M	(None)	Neuropsychological tests including a focused attention task	N.R.	RTD reduced response time in tasks requiring a response to incompatible stimuli (interpreted as an RTD-induced enhancement of attentional focus).
LEARNING AND MEMORY	Riedel et al. 1999	27 (15F, 12M)	(None)	Long-term and short-term memory tests in a neuropsychological test battery	67%	RTD selectively impaired performance on tests of long-term memory, but short-term memory and other functions were unperturbed. No differences between subjects with a positive family history versus negative FH.
FOOD CHOICES	Young et al. 1988	22M	(None)	Lunch selections in meal following RTD	72% (total TRP)	Used 50 g RTD. Following RTD, there were small increases in protein intake; no other effects on carbohydrate or fat or total caloric intake.
FOOD CHOICES	Oldman et al. 1994	12 F	POMS, VAS	Measured food taken in a meal following RTD	67%	Used 50 g RTD. No effects seen on hunger, mood, or food intake.
IMMUNE MEASURES	Ravindran et al. 1999	26 M	POMS	Immune measures: cytokine levels (IL-1 β , IL-6) and mitogen-stimulated responses	57%	50 g challenge. No effects on immune measures. Small decline in POMS-D score in 5 of 14 subjects.
PAIN MEASURES	Abbott et al. 1992	60 M	POMS, Addiction research center inventory (ARCI)	Tolerance and threshold to pain using cold pressor test	70%	No mood effects were seen. RTD did not affect subjects' tolerance to cold-pressor pain, nor did it affect pain threshold. However, RTD modified (i.e., enhanced) tolerance to pain following the co-administration of morphine.

Abbreviations: MAACL = multiple affective adjective checklist; VAS = visual analog scale; HDRS = Hamilton depression rating scale; POMS = profile of mood states.

Mathe 1999; Zhang et al. 1999; Holmes et al. 1998; Heuser et al. 1998). Therefore, somatostatin may be a peptide whose synthesis mediates the depression-inducing effects of RTD. Whether sudden changes in somatostatin levels can induce (temporary) depressive symptoms is unknown, but may be worthy of exploration. Other regulatory proteins that may contain TRP — neurotransmitter receptors, ion channels, enzymes involved with neurotransmitter synthesis and degradation — also affect neuronal function. These other possibilities have not been investigated with respect to RTD. The assumption remains that 5-HT is the most parsimonious TRP “metabolite” capable of producing the spectrum of RTD effects.

SUMMARY

Several issues are important to consider in RTD-induced depressive relapse. Medication status, duration of treatment, degree of remission, plasma TRP levels, gender, gastrointestinal side effects and individual predisposition all may play a role. These hypotheses are testable. More research may be needed regarding the reversal of antidepressant effect in SSRI/MAOI treated patients, and in OCD and panic disorder as well. There is circumstantial evidence supporting the notion of (temporary) decreased serotonin release from neurons following RTD. Effects of RTD on lowering plasma TRP are consistent and robust. The most dramatic symptoms tend to occur in partially remitted depressed patients on serotonergic pharmacotherapy. However, this has yet to be replicated by independent groups. With regard to other non-mood measures, certain measures presumably under serotonergic control (such as REM sleep regulation) may remain sensitive to RTD after full remission with SSRIs or MAOIs, though mood and other depressive symptoms may not.

RTD has helped to illuminate some of the enormous complexity of serotonin's role in human behavior. Its main advantage has been its comparative lack of long-term ill effects. There has been controversy about the ethical underpinnings of research “challenge” protocols in which patients are hypothesized to experience increased negative symptoms as a result of participation. The same concerns apply to these types of studies. To our knowledge, there have been no serious negative responses of the RTD protocol, such as suicide attempt or hospitalization, although it is possible that such events occur but are reported only to the local institutional review board. According to published reports, when patients are withdrawn from RTD studies it is primarily due to gastrointestinal side effects, i.e., nausea, vomiting, or diarrhea. Perhaps there is a need to develop a central oversight committee or reporting center, such as those used in multi-site clinical trials, to keep track of

infrequent but serious side effects in challenge studies like RTD, so that the risk: benefit ratio may be evaluated more critically.

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