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# Mice Lacking Brain-Derived Serotonin Have Altered Swallowing Function

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## Abstract

The intricate sensorimotor neural circuits that control swallowing are heavily reliant on serotonin (5-hydroxytryptamine [5-HT]); however, the impact of 5-HT deficiency on swallow function remains largely unexplored. We investigated this using mice deficient in tryptophan-hydroxylase-2 (TPH2), the enzyme catalyzing the rate-limiting step in 5-HT synthesis. Videofluoroscopy was utilized to characterize the swallowing function of TPH2 knockout (*TPH2*$^{-/-}$) mice as compared with littermate controls (*TPH2*$^{+/+}$). Results showed that 5-HT deficiency altered all 3 stages of swallowing. As compared with controls, *TPH2*$^{-/-}$ mice had significantly slower lick and swallow rates and faster esophageal transit times. Future studies with this model are necessary to determine



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if 5-HT replacement may rescue abnormal swallowing function. If so, supplemental 5-HT therapy may have vast applications for a large population of patients with a variety of neurologic disorders resulting in life-diminishing dysphagia, particularly amyotrophic lateral sclerosis and Parkinson's disease, for which 5-HT deficiency is implicated in the disease pathogenesis.

### Keywords

serotonin deficiency; 5-hydroxytryptamine (5-HT); tryptophan hydroxylase (TPH); mouse model; videofluoroscopy; amyotrophic lateral sclerosis (ALS)

Dysphagia is a devastating symptom of many neurologic diseases and is often associated with complications such as malnutrition, aspiration pneumonia, decreased quality of life, and increased risk of mortality. Although the pathophysiology of dysphagia for each disease remains largely unknown, there is growing evidence that the intricate sensorimotor neural circuits involved in swallowing rely on the neurotransmitter serotonin (5-hydroxytryptamine [5-HT]).[1–3] In particular, the nucleus tractus solitarius (NTS) within the brainstem central pattern generator for swallowing contains a large distribution of $5\text{-HT}_{1a}$ receptors, suggesting that serotonergic neurons play a critical role in initiating swallowing.[3] However, this role remains highly controversial, as previous studies found inhibitory,[3] excitatory,[4,5] and negligible[6] actions of 5-HT in the NTS. In addition, the brainstem motor nuclei (ie, trigeminal, facial, nucleus ambiguus, dorsal motor nucleus of the vagus, and hypoglossal) that innervate the muscles involved in swallowing receive dense 5-HT innervation from the NTS and numerous other regions of the brain to facilitate the coordinated, sequential motor pattern of swallowing.[7,8] This wide-spread serotonergic influence suggests that 5-HT deficiency may influence swallow function.

Previous studies utilized pharmacologic manipulation of the serotonergic system to investigate the effect of 5-HT on swallowing. However, a genetically modified animal model, particularly one in which central 5-HT is lacking, could provide the best evidence that 5-HT is necessary for the complex neural control of swallowing. A potential target is tryptophan hydroxylase (TPH), which is the rate-limiting enzyme for 5-HT synthesis from tryptophan that exists as 1 of 2 isoenzymes: TPH1 or TPH2.[9,10] While TPH1 is primarily expressed in gastrointestinal tissue, TPH2 synthesizes 5-HT in the central nervous system.[9,10] Here, we used TPH2 knockout ($TPH2^{-/-}$) mice[9,11] and videofluoroscopy to investigate the impact of central nervous system–derived 5-HT deficiency on swallowing.

## Methods

### Animals

$TPH2^{-/-}$ mice[9,11] (n = 19: 9 females, 10 males) and littermate controls ($TPH2^{+/+}$; n = 25: 13 females, 12 males) were used for this study. Pups were generated from heterozygous breeders maintained on a mixed C57BL/6J and 129/SvJ genetic background; heterozygous offspring ($TPH2^{+/-}$) were excluded. Mice were group-housed by sex on a 12:12-hour light cycle with free access to food and water, except during swallow testing. All experiments were approved by the University of Missouri Institutional Animal Care and Use Committee.

### Videofluoroscopic Swallow Study

We used our custom low-energy fluoroscope and established videofluoroscopic swallow study (VFSS) protocol[12,13] to assess the swallowing function of each mouse at 6 and 12 months of age, representing young versus older adult mice prior to the onset of age-related swallowing impairment (presbyphagia).[13] Briefly, after an overnight (~16 hour) water restriction, mice underwent videofluoroscopy in a custom chamber in lateral view while voluntarily drinking thin liquid contrast (chocolate-flavored iohexol) from a bowl. Fluoroscopic videos (30 frames per second) were digitally recorded when mice were observed drinking via a webcam. The oropharyngeal and esophageal stages of swallowing were recorded separately (Figure 1) by moving the mouse within the x-ray beam with a custom remote-controlled platform.

Videos (n = 176: 44 mice × 2 anatomic views × 2 time points) were analyzed frame by frame by 2 independent blinded reviewers with Pinnacle Studio 14 video editing software (Pinnacle Systems, Inc, Mountain View, California). For each fluoroscopic view (ie, oropharyngeal vs esophageal stage of swallowing), five 2-second episodes of uninterrupted drinking were analyzed per mouse to quantify the VFSS metrics defined in Table 1. Discrepancies (<10%) were subjected to group consensus to resolve reviewer error.

## Results

VFSS data were analyzed with a 3-factor (group, time point, sex) repeated measures analysis of variance design and Bonferroni post hoc comparisons ($P \leq .05$, 2-sided) with SAS 9.4 (SAS Institute, Cary, North Carolina). As shown in Figure 2, 5-HT deficiency affected all 3 stages of swallowing. Compared with controls, *TPH2*$^{-/-}$ mice had significantly slower lick and swallow rates at 6 and 12 months of age, providing evidence of chronic oral and pharyngeal dysphagia, respectively. Sex differences were identified only for lick rate, which was impaired earlier for *TPH2*$^{-/-}$ males. The esophageal stage was affected only at 12 months of age, with *TPH2*$^{-/-}$ mice having significantly faster esophageal transit times as compared with controls. However, within-group comparisons over time revealed statistically longer esophageal transit only for controls, indicative of age-related effects. Indeed, significant age-related changes were observed for both groups, which was earlier than expected based on our previous work with a mouse model of primary aging.[13] No other health- or weight-related issues were experienced by either group.

## Discussion

Results from this study showed that mice lacking central 5-HT have altered swallowing function as compared with controls. Specifically, 5-HT deficiency caused reduced lick and swallow rates, which is in agreement with other studies showing that 5-HT in the NTS has an excitatory/facilitatory effect on oropharyngeal swallowing.[4,5] In addition, lick rate was impaired earlier for *TPH2*$^{-/-}$ males, which supports previous findings of sex differences in the 5-HT system of the brain.[14,15] *TPH2*$^{-/-}$ mice failed to show age-related changes in esophageal transit times, congruent with previous research showing that low levels of 5-HT potentiate spontaneous contractions in the esophagus.[16] However, it is unclear if faster esophageal transport in old age could be clinically beneficial versus a sign of dysphagia.

Future work is needed to investigate the corresponding mechanisms of action for these findings, including any modulating effect of 5-HT deficiency on other neurotransmitter systems involved in swallowing, such as dopamine and glutamate.

In conclusion, 5-HT replacement or selective serotonin reuptake inhibitors, such as paroxetine or fluoxetine, may have vast applications for a large population of patients with a variety of neurologic disorders resulting in life-diminishing dysphagia. For example, 5-HT deficiency has been implicated in amyotrophic lateral sclerosis[17–19] and Parkinson's disease,[20–22] both of which result in debilitating dysphagia. Furthermore, selective serotonin reuptake inhibitors have already shown promise in improving the swallowing function of patients after a stroke.[23] Therefore, early 5-HT supplementation may alleviate or delay symptoms of dysphagia in neurologic diseases, providing patients with a better quality of life and a reduced risk of mortality.

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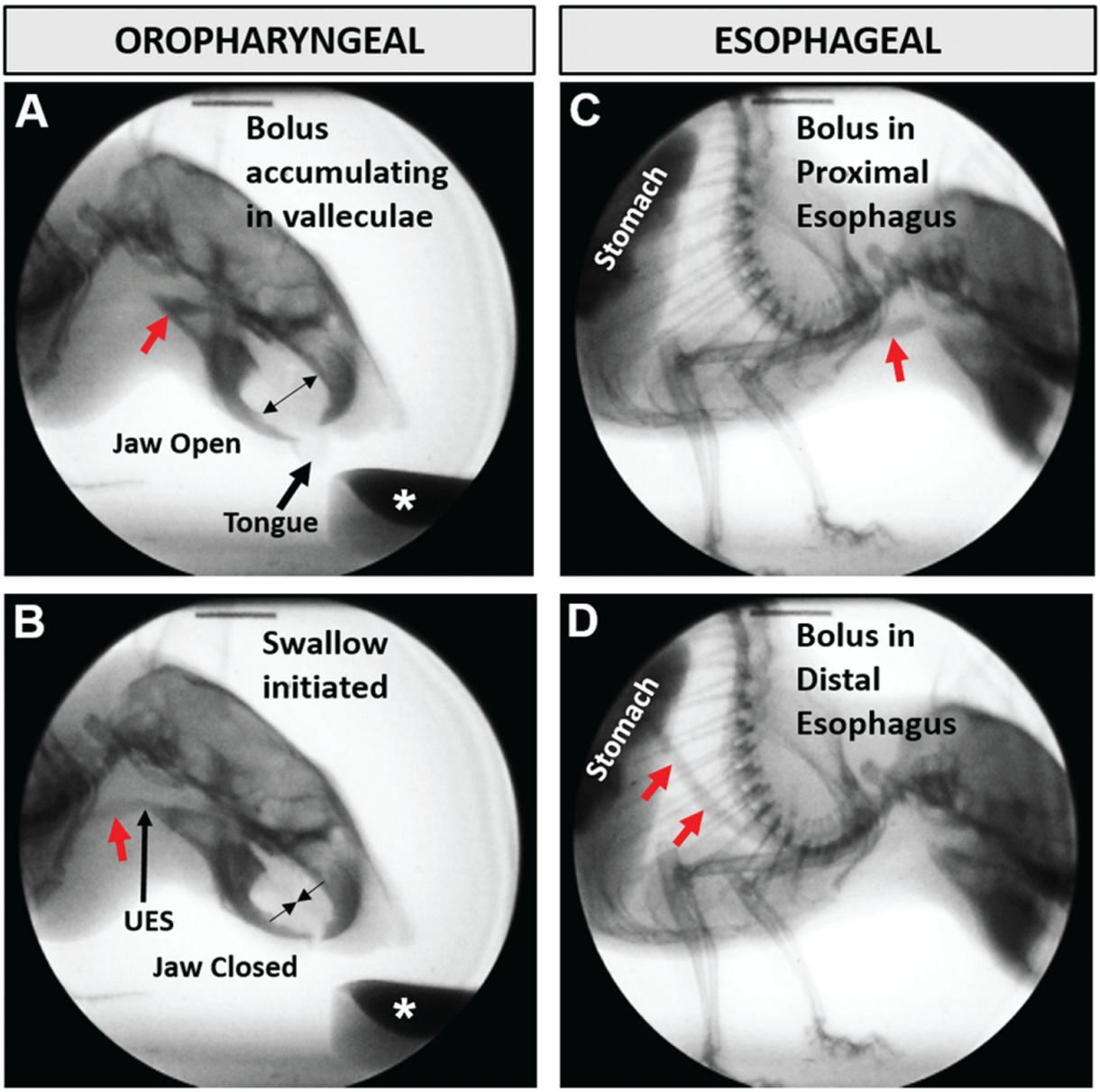


**Figure 1.**

Videofluoroscopic images of a mouse drinking contrast solution (asterisk). A bolus (red arrow) is tracked through the (A, B) oropharyngeal and (C, D) esophageal stages of swallowing. UES, upper esophageal sphincter.

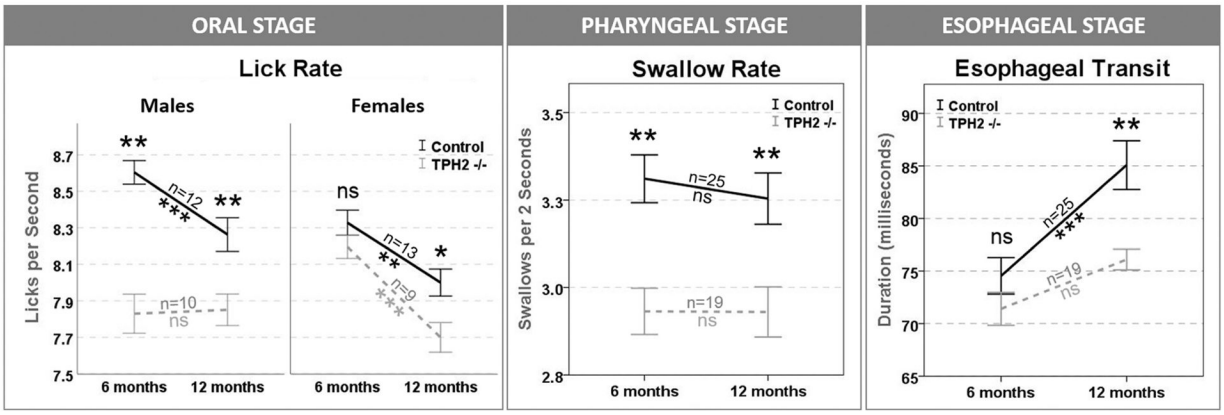


**Figure 2.**

5-Hydroxytryptamine deficiency affected all 3 stages of swallowing. Lick rate was impaired earlier for *TPH2*$^{-/-}$ males. *$P$ <.05. **$P$ <.01. ***$P$ ≤ .001. ns, nonsignificant; TPH2, tryptophan-hydroxylase-2. Error bars indicate standard error.

**Table 1.**

VFSS Metrics for Objective Quantification of Dysphagia in Mice.

| Stage of Swallowing | VFSS Metrics | Operational Definitions |
|---|---|---|
| Oral | Lick rate | The number of lick cycles per second (30 frames) during uninterrupted drinking, starting with the jaw at maximum open position and the tongue protruded. |
| Pharyngeal | Swallow rate | The number of swallows occurring during each 2-second (60 frames) episode of uninterrupted drinking, beginning at the rest frame immediately preceding bolus transit from the valleculae (ie, anatomic swallow trigger point in mice). |
| Esophageal | Esophageal transit time | The time (in milliseconds) that it takes the bolus to be swallowed from the proximal esophagus into the stomach. |

Abbreviation: VFSS, videofluoroscopic swallow study.