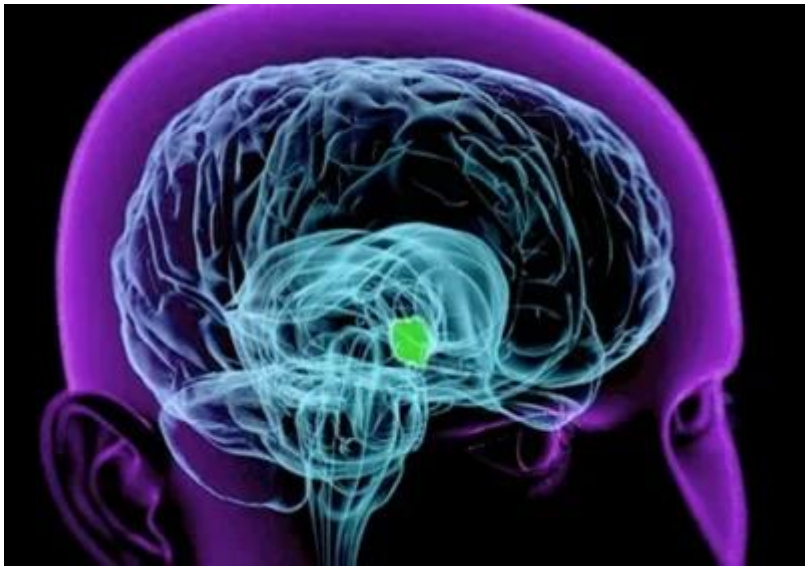
A significant proportion of male COVID-19 patients have low testosterone levels, which can persist for months after recovery from the infection. It is uncertain whether gonadotropin-releasing hormone (GnRH) neurons or their functions are affected in individuals infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The research group from France, the United Kingdom, Spain, Germany, Hungary, and Italy examined the hormone profile of male COVID-19 patients at various times after infection. They also examined possible SARS-CoV-2 infection of GnRH neurons and olfactory epithelia in the *postmortem* brain tissue samples of patients who died of COVID-19. The results have shown that persistent hypotestosteronemia in COVID-19 or long COVID syndrome could be of hypothalamic origin due to impaired GnRH function.

SARS-CoV-2 is an enveloped, positive-sense, single-stranded RNA virus. Its genome encodes four structural proteins, namely the spike (S), envelope (E), nucleocapsid (N), and membrane (M) proteins. Two host-cell factors are important for SARS-CoV-2 viral entry into many cell types: angiotensin-converting enzyme 2 (ACE2), which is bound by S protein, and transmembrane protease, serine 2 (TMPRSS2), which cleaves S protein, allowing this binding to take place.



Neurons secreting GnRH are located in the infundibular nucleus of the tuberal region of the hypothalamus. Their terminals contact fenestrated vessels of the pituitary portal system to secrete the hormone. A specialized population of hypothalamic glia, the tanycytes, controls this process. Tanycytic endfeets are in contact with fenestrated capillaries at the external pial surface of the median eminence, and dynamically interact with GnRH axon terminals, thereby regulating the periodic release of GnRH. This process may be disturbed in infected

patients, contributing to dysregulation of the hypothalamic-pituitary-gonadal (HPG) axis.

During embryogenesis, the GnRH neurons migrate from the olfactory placode into the brain but remain in contact with the olfactory bulb *via* long dendrites. Therefore, GnRH neurons can be infected by SARS-CoV-2 *via* two distinct neuroinvasive pathways, the olfactory pathway and the hematogenic pathway at the median eminence level, which the BBB does not completely protect.

Certain structures of the central nervous system (CNS), such as the choroid plexus and the circumventricular organs including the hypothalamus, are not completely protected by the blood-brain barrier (BBB) and can serve as virus entry points. The absence of the BBB in the median eminence- an adaptation that is essential for peptide neurohormones such as GnRH to reach their target cells in the pituitary gland and for circulating peripheral signals to enter the brain — may represent a gap in the brain's defense mechanisms against pathogens. In addition, the breakdown of the BBB and blood-CSF barrier, induced by systemic viral infection, facilitates the passage of viruses through the fenestrations into the CNS. The role of the hypothalamic circuits in the SARS-CoV-2 infection of the CNS, and their possible involvement in the neurological manifestations of COVID-19, has been discussed in previous papers.
https://discovermednews.com/the-involvement-of-hypothalamic-circuits-in-sars-cov-2-infection-of-the-central-nervous-system/

When the HPG axis is functioning correctly, GnRH triggers the release of gonadotropins, luteinizing hormone (LH), and follicle-stimulating hormone (FSH), which act on the gonads to stimulate the production of steroid hormones and gametogenesis. The feedback from gonadal steroids influences the release of GnRH and gonadotropins. A decrease in gonadal steroid levels leads to a compensatory increase in LH and FSH levels, and *vice versa*. The impaired function of GnRH leads to low testosterone levels or hypogonadotropic hypogonadism.

About the study

The study included 60 male patients, aged 35–82 years, positive for SARS-CoV-2, and 50 controls aged 28–87 years, uninfected with SARS-CoV-2. COVID-19 diagnosis was confirmed with positive reverse transcription polymerase chain reaction (rt-PCR) of nasopharyngeal swabs for SARS-CoV-2 or a positive serum SARS-CoV-2 IgG antibody test, chest radiograph, or computed tomography scan. The study also included 47 individuals diagnosed with post-COVID syndrome, older than 18 years, at least three months after COVID-19 diagnosis.

Individuals taking steroids after recovery from COVID-19 were excluded.

Blood samples were taken at the first week following admission, and at two and four weeks if the patient was still hospitalized. In 22 participants, serum testosterone (T), LH, and FSH levels were measured again over a year after acute COVID-19.

The researchers also examined possible SARS-CoV-2 infection of GnRH neurons and olfactory epithelia in the *postmortem* brain tissue samples of four patients who died of COVID-19 in the ICU, including one who had a viremia at the time of death. The control brain samples were from five age-matched patients who died before the pandemic or were not positive for SARS-CoV-2. The olfactory epithelia of 7-, 11- and 14-week-old human fetuses (from voluntarily terminated pregnancies) were also examined.

Results

The hormone profile of COVID-19 patients

During the first week in the ICU, testosterone levels were either moderately or severely decreased in 57 of 60 COVID-19 patients. The remaining 3 patients had normal or near-normal T levels and intermediate concentrations of LH.

Out of 57 patients with low testosterone levels, only 6 had the compensatory increase in the LH, as expected with the normal function of the HPG axis. 38 COVID-19 patients had intermediate LH concentrations, and 13 had low LH concentrations, which indicates dysfunction of the HPG axis. The FSH levels were similar to LH.

More than a year after acute COVID-19, in 7 individuals who had dysfunction of the HPG axis at the first follow-up, testosterone levels were normal, and in 2 individuals testosterone levels were extremely low without a compensatory increase in LH. These results confirm that hypogonadism in these individuals could be of hypothalamic origin.

A comparable analysis of patients diagnosed with long COVID syndrome showed low low testosterone levels in 47 long COVID patients. Importantly, in 11 of them, there was no increase in LH, suggesting a persistent dysfunction of the HPG axis. FSH levels were mostly normal.

The examination of postmortem brain tissue samples

One-third of GnRH neurons from the hypothalamus of four patients who died of COVID-19 in

the ICU had a bloated or abnormal morphology. In contrast, the number of abnormal GnRH neurons was negligible in the uninfected controls.

The SARS-CoV-2 N protein was detected in the hypothalamus of three patients who died of COVID-19, including one with viremia. Multifunctional hypothalamic glia, called tanycytes, were infected with the SARS-CoV-2. Furthermore, the SARS-CoV-2 N protein and double-stranded RNA were abundant in the median eminence/infundibular nucleus, indicating robust viral entry and replication. In contrast, SARS-CoV-2 was absent in control brain samples from five age-matched patients who died before the pandemic or were not positive for SARS-CoV-2.

The SARS-CoV-2 S protein was found in vessels, some neuron-like cells, and cells of the ependymal wall. The S protein levels were extremely high in tanycytic endfeets that co-express ACE2-and TMPRSS2. The S protein, ACE2, and TMPRSS2 were also abundant in the olfactory nerve layer, where axons from sensory neurons of the olfactory epithelium enter the olfactory bulb. Numerous olfactory bulb cells bordering the olfactory nerve layer were positive for SARS-CoV-2 double-stranded RNA.

The *postmortem* examination of olfactory sensory neurons from 7-, 11- and 14-week-old human fetuses revealed abundantly expressed ACE2 and TMPRSS2. The authors hypothesized that in human fetuses some GnRH neurons can be infected with the SARS-CoV-2 *via* the olfactory pathway during embryonic development.

Conclusion

This study has shown that infection with SARS-CoV-2 may result in persistent or delayed hypogonadotropic hypogonadism in acute COVID-19 patients and individuals diagnosed with long COVID syndrome. *Postmortem* examination of hypothalamic tissue revealed that one-third of GnRH neurons from the hypothalamus of patients who died of COVID-19 had abnormal morphology. The SARS-CoV-2 infected and replicated in multifunctional hypothalamic glia, called tanycytes, which regulate the periodic release of GnRH, and the median eminence/infundibular nucleus, which the BBB does not completely protect.

This article was published in eBioMedicine.

Journal Reference

Suave F, Nampoothiri S, Clarke SA et al. Long-COVID cognitive impairments and reproductive hormone deficits in men may stem from GnRH neuronal death. EbioMedicine

2023; 96: 104784. https://doi.org/10.1016/j.ebiom.2023.104784