DOI 10.1007/s11055-018-0645-x
Neuroscience and Behavioral Physiology, Vol. 48, No. 7, September, 2018

Expression of mGluR2/3 Metabotropic Glutamate Receptors in the Ventrolateral Part of the Solitary Tract Nucleus in Rats During the Early Postnatal Period in Health and in Prenatal Serotonin Deficiency

L. I. Khozhai

UDC 611.818.6:65:599.323.4

Translated from Morfologiya, Vol. 152, No. 6, pp. 11–16, November–December, 2017. Original article submitted May 19, 2017. Revised version received October 16, 2017.

Objective. to study the dynamics of the expression of mGluR2/3 metabotropic receptors in the ventral and lateral respiratory subnuclei of the solitary tract nucleus (STN) in the early postnatal period in health and after prenatal serotonin deficiency in rats. **Materials and methods.** Studies were performed on Wistar laboratory rats (n = 19). Tryptophan hydroxylase was inhibited using parachlorophenylalanine. The ventral and lateral respiratory subnuclei of the STN were studied on days 5, 10, and 20. An immunohistochemical method was used to study the distribution of mGluR2 and mGluR3 metabotropic receptors (mGluR2/3). **Results.** During the first postnatal week, both subnuclei showed high levels of mGluR2/3 expression. During the second week, there was a sharp reduction in mGluR2/3 expression, followed by an increase by the end of the third week. Decreases in serotonin content during the prenatal period affected the intensity of mGluR2/3 expression in both subnuclei of the STN. During the early postnatal period, there was a significant reduction (more than two-fold) in the expression of mGluR2/3 expression at all time pints, this being more marked in the ventral subnucleus. **Conclusions.** Changes in the level of expression of mGluR2/3 metabotropic glutamate receptors during the early postnatal period occurred in the ventrolateral part of the STN. Serotonin deficiency led to a sharp reduction in the expression of mGluR2/3 receptors in the respiratory subnuclei during the early postnatal period.

Keywords: brain, solitary tract nucleus, respiratory subnuclei, metabotropic glutamate receptors, serotonin.

Integration of information arriving in the solitary tract nucleus (STN) from the visceral organs (respiratory, cardiovascular, etc.) is mediated by classical neurotransmitters, the most important excitatory neurotransmitter being glutamate [1, 3]. Glutamate release and spike transmission are regulated by different transmitters and their receptor systems – glutamate, GABA, and serotonin [10]. Glutamate transmission in the CNS can either increase or decrease depending on activation of specific receptors, especially particular subtypes of metabotropic glutamate receptors (mGluR). In the STN, these receptors subtypes are present in synapses and are widely distributed both on presynaptic terminals of afferent fibers and on neuron bodies. Metabotropic glutamate receptors are divided into three groups on the basis of amino acid sequence homology. The mGluR2 and mGluR3 (mGluR2/3) subtypes are in group II, which are located predominantly on presynaptic terminals, are coupled with adenylate cyclase, and are autoreceptors, inhibiting glutamate release by blocking potential-dependent calcium ion channels, which significantly decreases the synaptic transmission of glutamate [4, 15, 19].

mGluR2/3 are believed to function as modulators of glutamate transmission, regulating transmitter release, and activation of these receptors, in contrast to their ionotropic analogs, does not lead to rapid electrophysiological responses [6]. This mediation and control of excitatory transmitter release in the STN by this receptor network plays an

Laboratory for Nervous System Ontogeny, Pavlov Institute of Physiology, Russian Academy of Sciences, St. Petersburg, Russia; e-mail: astarta0505@mail.ru.

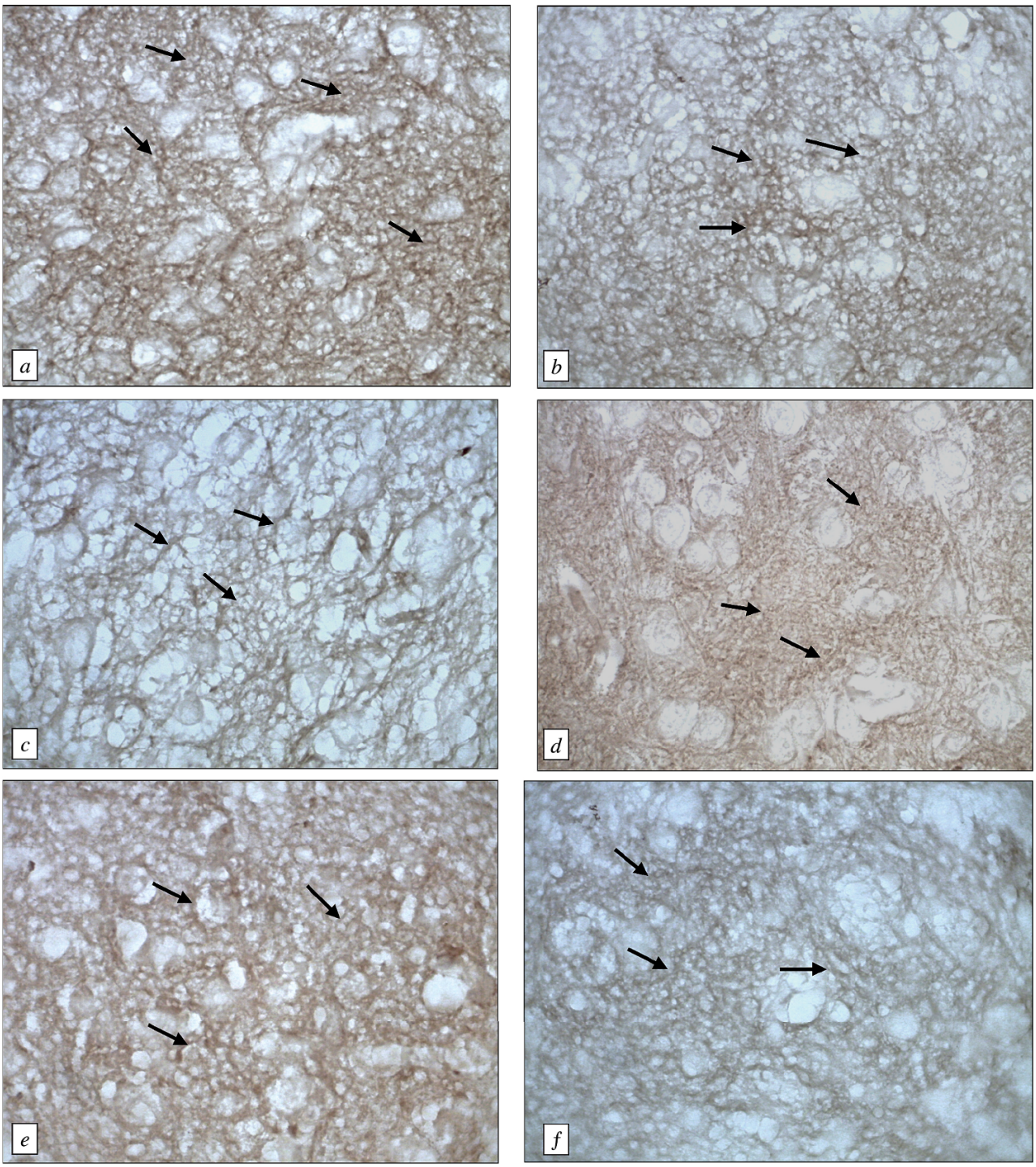

Fig. 1. Solitary tract nucleus in rats (lateral subnucleus) at different time points of postnatal development in controls (*a*, *c*, *e*) and in conditions of prenatal serotonin deficiency (*b*, *d*, *f*). *a*, *b*) day 5; *c*, *d*) day 10; *e*, *f*) day 20. Reaction product distribution density (arrows) in the processes and terminals in the neuropil was greater in control rats than experimental animals. Immunocytochemical reaction for metabotropic glutamate receptors (mGluR2/3). Objective ×100. Ocular ×10.

important role in the mechanisms balancing excitation and inhibition of neurons in the respiratory subunits of the STN (during processing of incoming information), especially in the early postnatal period of development (i.e., during the maturation of the respiratory system in mammals and humans), when pathological changes can arise in the structure of the respiratory nuclei, leading to the development of respiratory dysfunction. Despite this, researchers have

paid virtually no attention to changes in the expression of mGluR2/3 metabotropic receptors in the respiratory subunits of the STN in the early postnatal period.

Serotonin (5-HT), along with other transmitters, is involved in glutamate neurotransmission from visceral afferents in the STN [18]. The subnuclei of the STN contain significant numbers of serotoninergic fibers running from the medullary raphe nuclei [20]. Constant spontaneous 5-HT release from the terminals of serotoninergic fibers has been shown to occur in this location, neurotransmission by this transmitter being regulated by the reuptake system [7]. 5-HT receptors, located mainly presynaptically on afferent terminals, are intensely expressed in the STN – particularly the 5-HT_3 and 5-HT_{1A} subtypes) [11, 12, 17]. Serotonin, acting via activation of these receptor subtypes, promotes either glutamate release or the inhibition of its release [10]. Interactions between serotonin and glutamate and their receptor systems have received little attention in the literature, and the question of the influence of serotonin deficiency on the activity of the excitatory glutamatergic system in the respiratory nuclei in early postnatal development remains unanswered.

Thus, the aim of the present work was to study the dynamics of changes in the expression of mGluR2/3 metabotropic receptors in the ventral and lateral respiratory subnuclei of the STN during the early postnatal period in health and prenatal serotonin deficiency in rats.

Materials and Methods. Experiments were performed on Wistar laboratory rats (n = 16) from the animal house of the Institute of Physiology, Russian Academy of Sciences. Animal keeping and all experimental procedures were carried out in compliance with the "Regulations for Studies Using Experimental Animals" (USSR Ministry of Health Decree No. 755 of August 12, 1977). Endogenous serotonin levels were decreased by inhibition of tryptophan hydroxylase (the enzyme synthesizing serotonin) with p-chlorophenylalanine (PCPA) (Sigma, USA). Female rats received PCPA (400 mg/kg) i.p. on day 9 of pregnancy (to obtain prolonged decreases in serotonin of up to 50–80% during the period in which the STN, solitary tract, and serotoninergic system form in fetuses). The medulla oblongata in developing rat pups was studied on days 5, 10, and 20. Controls were animals of the same periods of development obtained from intact females. Studies were performed using groups of 5–6 experimental and control rat pups of each developmental period. Specimens were fixed in zinc-ethanol-formaldehyde in phosphate-buffered saline pH 7.4 and embedded in paraffin using standard methods; serial transverse sections of the medulla oblongata of thickness 5 μm were cut at the level of the bregma 11.88–12.00 [16].

Polyclonal rabbit antibodies to glutamate receptors (mGluR2/3) (Abcam, USA) were used to detect the distribution of mGluR2 and mGluR3 (mGluR2/3) metabotropic receptors. Secondary reagents for mGluR2/3 were from an EnVision+ System-HRP Labelled Polymer Anti-Rabbit kit (DakoCytomation, USA). Reaction product was visualized with the chromogen DAB+ (Fako, Denmark). The cellular localization of mGluR2/3 is that of an integral membrane protein. Immunocytochemical reactions in control and experimental animals were performed simultaneously and some sections were counterstained with thionine (Serva, USA, Germany) and embedded in synthetic Permount medium (Termo, USA).

Morphological analysis was performed using digital images of serial sections obtained using a Leica DME light microscope (Leica, Germany) and a Leica EC3 digital camera (Leica, Germany). The density of plexuses of immunopositive terminal processes in the neuropil and the density of grains and large granules (the latter are presumptively terminal synaptic structures and their groupings) were counted in standard areas of 0.1 mm^2 [9]. Reaction product optical density in the neuropil was assessed using images obtained with a digital video camera and VideoTest Master Morphology software (Video Test, St. Petersburg). Statistical analysis was run by ANOVA (Statistica 7.0, Statsoft Inc., USA). Arithmetic means and standard errors were calculated. The critical level of significance was $p < 0.05$.

Results. *Lateral subnucleus of the STN*. On day 5, the neuropil in control animals showed intense staining of processes and terminals forming a dense network containing many immunopositive granules (Fig. 1, *a*) (the optical density of the reaction product was 0.097 ± 0.008). On day 10, the staining intensity of processes and terminals decreased from that on day 5 (0.050 ± 0.006). The density of immunopositive processes decreased and these formed a looser network (see Fig. 1, *c*). On day 20, the staining intensity of processes and granules in the neuropil increased again (see Fig. 1, *e*), and the distribution density of immunopositive processes also increased (0.088 ± 0.008).

On day 5, immunopositive processes and terminals in the lateral subnucleus of experimental animals, as opposed to those in control animals, formed a loose network (see Fig. 1, *b*), with lower staining intensity (0.050 ± 0.005). On day 10, both the staining intensity of processes and terminals and the density of the network of processes (0.040 ± 0.008) in the neuropil decreased, though many immunopositive granules remained (see Fig. 1, *d*). On day 20, the staining intensity of processes and terminals again increased (see Fig. 1, *f*) but was less than that in control animals; the density of processes was also lower (0.043 ± 0.004).

Ventral subnucleus of the STN. On day 5, the neuropil of ventral subnucleus in control animals, like the neuropil of the lateral subnucleus, showed intense staining of processes and terminals, forming a dense network (optical density 0.092 ± 0.005). On day 10, staining intensity decreased and there were significantly fewer immunopositive processes and terminals in the neuropil (0.054 ± 0.007), forming a loose network. On day 20, staining intensity again increased, as did the density of the network of immunopositive processes, terminals, and granules (0.089 ± 0.006).

On day 5, the staining intensity of processes and terminals forming a loose network in the ventral subnucleus of experimental animals was lower than that in control animals. Optical density was 0.049 ± 0.005. On day 10, there was some decrease in the staining intensity of processes and terminals, processes formed a loose network, and there were many small granules. Optical density was 0.039 ± ± 0.003. On day 20, the staining intensity of the network of processes and terminals again increased, as did optical density (0.0424 ± 0.006).

Discussion. These studies showed that during early postnatal development, control animals showed changes in the level of expression of mGluR2/3 metabotropic glutamate receptors. During the neonatal period, both the ventral and the lateral subnuclei showed high levels of expression of mGluR2/3. Increases in postnatal age (during the second week) were accompanied by a sharp decrease in mGluR2/3 expression, though by juvenile age (the end of week 3) the expression level again increased in both respiratory subnuclei. Metabotropic glutamate receptors are known to be members of the G-protein-coupled receptor family and form three groups including eight subtypes. Group I includes the mGluR1 and mGluR5 subtypes – these receptors promote release of glutamate and are located on neuron bodies. Receptors of group II (mGluR2 and mGluR3) and group III (mGluR4, mGluR6, mGluR7, and mGluR8) are autoreceptors, excitation of which suppresses glutamate release, and are mainly located on presynaptic membranes [4, 15, 19]. Decreases in glutamate release are mediated by the presynaptic receptor element [5, 8, 14, 21]. Confirmation of this may be provided by data showing that agonists of group II metabotropic receptors decrease the synaptic transmission of glutamate in baroreceptor synapses in the STN while, conversely, blockade of group II receptors increases synaptic glutamate transmission [13]. These observations and the results obtained in our studies suggest strongly that during the neonatal period, when levels of mGluR2/3 expression are quite high, glutamate release should be low, though the sharp decrease in mGluR2/3 expression during the second postnatal week may promote increases in its release, and the increase in receptor expression during the juvenile period (i.e., by the end of the third week) should again inhibit glutamate release. As neurons in the ventral and lateral subnuclei of the STN are mainly inspiratory [2], the early postnatal period and juvenile age are probably critical in relation to the decrease in the level of the excitatory neurotransmitter and the possible increase in inhibitory effects in the respiratory subnuclei of the STN at this time. The source of constant serotonin release in the STN subnuclei is known to consist of a significant number of fibers running from the medullary raphe nuclei [8, 21]. The STN contains intense expression of 5-HT receptors (mainly the 5-HT_3 and 5-HT_{1A} subtypes), which are mostly located presynaptically on afferent terminals [12, 15, 17]. There are two pathways for the actions of serotonin - excitatory and inhibitory. Operating via activation of these receptor subtypes, serotonin promotes either the release of glutamate (activation of 5-HT_3) or its inhibition (activation of 5-HT_{1A}) [10]. Release of large quantities of serotonin in the STN from serotoninergic fibers, activating the receptor component, may be involved in glutamate neurotransmission [10].

The results obtained here showed that decreases in serotonin content during the prenatal period affected the intensity of expression of mGluR2/3 in both subunits of the STN. More than twofold reductions in mGluR2/3 receptor expression at all study time points during the early postnatal period were more marked in the ventral subnucleus. This change in mGluR2/3 receptor expression would probably promote increases in glutamate release in the respiratory subunits and the resulting increase in the excitability of inspiratory neurons, leading to impairment in the balance between excitation and inhibition of STN neurons during the respiratory cycle. The likely explanation for the decreased expression of receptors is that serotonin deficiency can lead to changes in the processes underlying the synthesis of receptor proteins and the resulting impairment to the formation of glutamatergic synapses, which during the postnatal period may be responsible for the insufficient development of the excitatory glutamatergic receptor network overall. We cannot exclude the possibility that the prenatal decrease in the serotonin content may lead to changes in the structure of the medullary raphe nuclei during their development and establishment, resulting in not only a decrease in the number of serotonin fibers running to the STN, but also a secondary decrease in the level of serotonin released in the STN. The suggestion that prenatal serotonin deficiency may be responsible for impairment to the innervation of the respiratory organs, leading to a decrease in the number of afferent fibers running from them to the STN, seems very likely, though it does not exclude the possibility of a sharp decrease in the number of serotonin receptors located on their terminals and involved in glutamate neurotransmission.

These studies show that during the first three weeks of postnatal life, the respiratory subnuclei of the STN show oscillations in the level of expression of mGluR2/3 receptors suppressing glutamate release. Considering the ability of glutamate to activate different receptor types, this period of ontogeny probably involves tuning of neurons to transmission and realization of excitation, i.e., maturation of the glutamatergic system, subsequently supporting the concordant operation of all its elements. Serotonin-induced structural impairments of the glutamatergic receptor network may undoubtedly be the basis for the development of respiratory dysfunction during the postnatal period.

This study was supported by the Russian Foundation for Basic Research (Grant No. 15-04-02167).

The author has no conflict so interests.

REFERENCES

1. M. C. Andresen and D. L. Kunze, "Nucleus tractus solitarius – gateway to neural circulatory control," *Annu. Rev. Physiol.*, **56**, 93–116 (1994).
2. A. Baude, C. Strube, F. Tell, and J. P. Kessler, "Glutamatergic neurotransmission in the nucleus tractus solitarii: structural and functional characteristics," *J. Chem. Neuroanat.*, **38**, 145–153 (2009).
3. A. C. Bonham and C. Y. Chen, "Glutamatergic neural transmission in the nucleus tractus solitarius: N-methyl-D-aspartate receptors," *Clin. Exp. Pharmacol. Physiol.*, **29**, No. 5–6, 497–502 (2002).
4. J. Cartmell and D. D. Schoepp, "Regulation of neurotransmitter release by metabotropic glutamate receptors," *J. Neurochem.*, **75**, 889–907 (2000).
5. C.-Y. Chen, J. M. Horowitz, and A. C. Bonham, "A presynaptic mechanism contributes to depression of autonomic signal transmission in NTS," *Am. J. Physiol.*, **277**, 1350–1360 (1999).
6. C. Y. Chen, E. H. Ling Eh, J. M. Horowitz, and A. C. Bonham, "Synaptic transmission in nucleus tractus solitarius is depressed by Group II and III but not Group I presynaptic metabotropic glutamate receptors in rats," *J. Physiol.*, **538**, 773–786 (2002).
7. L. C. Daws, "Unfaithful neurotransmitter transporters: focus on serotonin uptake and implications for antidepressant efficacy," *Pharmacol. Ther.*, **121**, 89–99 (2009).
8. R. B. Felder and C. M. Heesch, "Interactions in nucleus tractus solitarius between right and left carotid sinus nerves," *Am. J. Physiol.*, **253**, 1127–1135 (1987).
9. A. Guthmann, J. M. Fritschy, P. O. Ottersen, et al., "GABA, GABA transporters, GABA(A) receptor subunits and GAD mRNAs in the rat parabrachial and Kölliker-Fuse nuclei," *J. Comp. Neurol.*, **400**, No. 2, 229–243 (1998).
10. P. S. Hosford, S. W. Miffin, and A. G. Ramage, "5-Hydroxytryptamine–mediated neurotransmission modulates spontaneous and vagal-evoked glutamate release in the nucleus of the solitary tract effect of uptake blockade," *J. Pharmacol. Exp. Ther.*, **349**, No. 2, 288–296 (2014).
11. R. D. Jeggo, D. O. Kellett, Y. Wang, et al., "The role of central 5-HT_3 receptors in vagal reflex inputs to neurones in the nucleus tractus solitarius of anaesthetized rats," *J. Physiol.*, **566**, 939–953 (2005).
12. R. A. Leslie, D. J. M. Reynolds, P. L. R. Andrews, et al., "Evidence for presynaptic 5-hydroxytryptamine$_3$ recognition sites on vagal afferent terminals in the brainstem of the ferret," *Neuroscience*, **38**, 667–673 (1990).
13. Z. Liu, C.-Y. Chen, and A. C. Bonham, "Metabotropic glutamate receptors depress vagal and aortic baroreceptor signal transmission in the NTS," *Am. J. Physiol.*, **275**, 1682–1694 (1998).
14. S. W. Mifflin and R. B. Felder, "An intracellular study of time-dependent cardiovascular afferent interactions in nucleus tractus solitarius," *J. Neurophysiol.*, **59**, 1798–1813 (1988).
15. Y. Ohi, S. Kimura, and A. Haji, "Modulation of glutamatergic transmission by metabotropic glutamate receptor activation in second-order neurons of the guinea pig nucleus tractus solitarius," *Brain Res.*, **1581**, 12–22 (2014).
16. G. Paxinos and C. Watson, *The Rat Brain in Stereotaxic Coordinates*, Academic Press, Sydney (2004), 5th ed.
17. G. D. Pratt and N. G. Bowery, "The 5-HT_3 receptor ligand, [^{3}H]BRL 43694, binds to presynaptic sites in the nucleus tractus solitarius of the rat," *Neuropharmacology*, **28**, 1367–1376 (1989).
18. A. G. Ramage and C. M. Villalun, "5-Hydroxytryptamine and cardiovascular regulation," *Trends. Pharmacol. Sci.*, **29**, 472–481 (2008).
19. M. Scanziani, P. A. Salin, K. E. Vogt, et al., "Use-dependent increases in glutamate concentration activate presynaptic metabotropic glutamate receptors," *Nature*, **385**, 630–634 (1997).
20. N. Schaffar, J. P. Kessler, O. Bosler, and A. Jean, "Central serotonergic projections to the nucleus tractus solitarii: evidence from a double labeling study in the rat," *Neuroscience*, **26**, 951–958 (1988).
21. J. H. Schild, J. W. Clark, C. C. Canavier, et al., "Afferent synaptic drive of rat medial nucleus tractus solitarius neurons: Dynamic simulation of graded vesicular mobilization, release, and non-NMDA receptor kinetics," *J. Neurophysiol.*, **74**, 1529–1548 (1995).