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DEVELOPMENT OF EXCITATORY SYNAPTIC TRANSMISSION TO THE SUPERIOR PARAOLIVARY AND LATERAL SUPERIOR OLIVARY NUCLEI OPTIMIZES DIFFERENTIAL DECODING STRATEGIES

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Abstract—The superior paraolivary nucleus (SPON) is a prominent structure in the mammalian auditory brainstem with a proposed role in encoding transient broadband sounds such as vocalized utterances. Currently, the source of excitatory pathways that project to the SPON and how these inputs contribute to SPON function are poorly understood. To shed light on the nature of these inputs, we measured evoked excitatory postsynaptic currents (EPSCs) in the SPON originating from the intermediate acoustic stria and compared them with the properties of EPSCs in the lateral superior olive (LSO) originating from the ventral acoustic stria during auditory development from postnatal day 5 to 22 in mice. Before hearing onset, EPSCs in the SPON and LSO are very similar in size and kinetics. After the onset of hearing, SPON excitation is refined to extremely few (2:1) fibers, with each strengthened by an increase in release probability, yielding fast and strong EPSCs. LSO excitation is recruited from more fibers (5:1), resulting in strong EPSCs with a comparatively broader stimulus–response range after hearing onset. Evoked SPON excitation is comparatively weaker than evoked LSO excitation, likely due to a larger fraction of postsynaptic GluR2-containing Ca^{2+}-impermeable AMPA receptors after hearing onset. Taken together, SPON excitation develops synaptic properties that are suited for transmitting single events with high temporal reliability and the strong, dynamic LSO excitation is compatible with high rate-level sensitivity. Thus, the excitatory input pathways to the SPON and LSO mature to support different decoding strategies of respective coarse temporal and sound intensity information at the brainstem level.

Key words: auditory brainstem, superior olivary complex, cochlear nucleus, AMPA receptors, plasticity, acoustic streaming.

INTRODUCTION

The superior paraolivary nucleus (SPON) is a prominent mammalian auditory brainstem nucleus. Due to the high sensitivity of its neurons to abrupt sound transients and large fluctuations in sound amplitude over time (Kuwada and Batra, 1999; Behrend et al., 2002; Kulesza et al., 2003; Kadner and Berrebi, 2008; Felix et al., 2011, 2013), the SPON has been hypothesized to play a major role in processing natural sounds that are rhythmically modulated (Theunissen and Elie, 2014). The fact that the SPON is primarily driven by monaural sound stimulation (Kuwada and Batra, 1999; Kulesza et al., 2003) lends further support in favor of its involvement in the processing of communication sounds (Plomp, 1976; Culling et al., 2003). In contrast, the neighboring lateral superior olivary nucleus is known to process binaural sound cues, which are used to localize sound sources (Tollin, 2003). The underlying cellular mechanism for LSO sound processing is known to originate from integration of excitation from the anterior ventral cochlear nucleus (AVCN) and inhibition from the medial nucleus of the trapezoid body (MNTB) (Boudreau and Tsuchitani, 1968; Cant and Casseday, 1986; Sanes and Rubel, 1988; Wu and Kelly, 1992). Although the SPON has recently received increased attention, much less is known of how the inputs to its neurons contribute to sound processing compared to neighboring auditory nuclei.

SPON activity is characteristically triggered *in vivo* at the cessation of contralateral sound stimulation, generating an offset response (Kuwada and Batra, 1999; Behrend et al., 2002; Dehmel et al., 2002; Kulesza et al., 2003). This offset response has been linked to hyperpolarization-activated rebound spiking in SPON neurons *in vitro* (Felix et al., 2011; Kopp-Scheinpflug et al., 2011), triggered by feed-forward inhibition from the MNTB (Kuwabara et al., 1991; Kulesza et al., 2007; Kopp-Scheinpflug et al., 2011). In addition to the characteristic offset response, an onset response to contralateral sound stimulation has also been described for SPON neurons (Kuwada and Batra, 1999; Behrend et al., 2002; Dehmel et al., 2002; Felix et al., 2013), but despite the presence of a well-documented excitatory pathway from the posterior ventral cochlear

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Abbreviations: ACSF, artificial cerebrospinal fluid; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AMPAR, AMPA receptor; AVCN, anterior ventral cochlear nucleus; EPSC, excitatory postsynaptic current; EPSP, excitatory postsynaptic potential; GluR, glutamate receptor; IAS, intermediate acoustic stria; LSO, lateral superior olive; mEPSC, miniature EPSC; MNTB, medial nucleus of the trapezoid body; NMDA, N-methyl-D-aspartate; NMDAR, NMDA receptor; PPR, paired-pulse ratio; PVCN, posterior ventral cochlear nucleus; SPON, superior paraolivary nucleus; uEPSC, unitary EPSC; VAS, ventral acoustic stria.

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nucleus (PVCN) to the contralateral SPON via the intermediate acoustic stria (IAS; Zook and Casseday, 1985; Friauf and Ostwald, 1988; Thompson and Thompson, 1991; Schofield, 1995; Saldaña et al., 2009), the physiological implications of this projection on SPON function have not been explored. While there have been reports of some SPON responses that are not monaural or do not have offset spiking (Behrend et al., 2002; Dehmel et al., 2002), we focused the current study on neurons with contralaterally-driven offset responses because they have been well characterized, are present in all species studied thus far (bat: Grothe, 1994; rabbit: Kuwada and Batra, 1999; gerbil: Behrend et al., 2002; Dehmel et al., 2002; rat: Kulesza et al., 2003, 2007; Kadner and Berrebi, 2008; mouse: Felix et al., 2011; 2012; Kopp-Scheinpflug et al., 2011), and possess distinct properties that make them well suited for processing the identity of natural sound (Kadner and Berrebi, 2008; Felix et al., 2011).

One important question is to what extent the excitatory transmission to the SPON is developmentally regulated. For instance, it is possible that SPON excitation has a more prominent role before hearing onset, at ages when glutamatergic depolarization has been shown to drive refinement of the input circuitry in the adjacent LSO (Gillespie et al., 2005; Noh et al., 2010). In order to characterize the physiological properties of the PVCN excitatory projection to the SPON, we used whole-cell patch-clamp recordings obtained in mouse brain slices to study synaptically evoked excitation during auditory development. In addition to describing the developmental properties of SPON excitation for the first time, we systematically compared these properties to the well-characterized LSO excitation (Sanes and Rubel, 1988; Sanes, 1993; Wu and Fu, 1998; Case et al., 2011; Alamilla and Gillespie, 2011; Lee et al., 2016).

EXPERIMENTAL PROCEDURES

Animals and the preparation

Experimental procedures are in accordance with the EC Council Directive (86/89/ECC) and have been approved by the local Animal Care and Use Committees in Sweden (Permit N32/13). Briefly, the animals were decapitated under anesthesia in conformity with the rules set by the EC Council Directive (86/89/ECC) and the brainstem was carefully removed and placed in ice-cold low sodium, high sucrose artificial cerebrospinal fluid (aCSF, see below). Transverse slices of the brainstem area of the superior olivary complex were prepared from CBA mice aged P5–22 with a vibratome (VT1200; Leica, Wetzlar, Germany) and incubated at 32 °C in normal aCSF (see below) for 20–60 min, after which they were allowed to cool to room temperature. Recordings were obtained within 4–5 h of the preparation. To minimize the loss of synaptic inputs in the preparation of slices, we investigated the trajectory of the CN projection with immunohistochemistry (Adams, 1997) prior to the experiments. The brain was accordingly blocked at ~20° caudal-rostral angle at the mid-cortex level and slices were cut with a thickness of 170–200 μm from the caudal brainstem. This preparation technique preserved both the intermediate and the ventral acoustic stria (VAS) in the same plane.

Solutions and pharmacology

The low sodium, high sucrose aCSF contained (in mM): 85 NaCl, 2.5 KCl, 1.25 NaH2PO4, 25 $NaHCO_3$, 75 sucrose, 25 glucose, 0.5 $CaCl_2$, 4 $MgCl_2$ and 0.5 ascorbic acid, whereas the normal aCSF contained (in mM): 125 NaCl, 2.5 KCl, 1.25 $NaH2PO_4$, 26 $NaHCO_3$, 25 glucose, 2 $CaCl_2$ and 1 $MgCl_2$. The external solutions were bubbled continuously with carbogen gas (95% O_2–5% CO_2), generating a pH of 7.4.

For current clamp recordings, the internal pipette solution contained (in mM): 130 K-gluconate, 5 KCl, 10 HEPES, 1 EGTA, 2 Na_2-ATP, 2 Mg-ATP, 0.3 Na_3-GTP, 10 Na_2-phosphocreatinine, adjusted to pH 7.3 with KOH. For voltage clamp recordings, the Cs-based internal solution contained (in mM): 130 $CsMeSO_4$, 10 HEPES, 1 EGTA, 5 NaCl, 1 $CaCl_2$, 2 Mg-ATP, 0.3 Na_3-GTP, 10 Na_2-phosphocreatinine, adjusted to pH 7.3 with CsOH. QX-314 (5 mM) was added to the internal solution in experiments that used a stimulus electrode to evoke excitatory responses in order to obviate action potentials. Liquid junction potentials of 11.6 mV (K-based solution) and 0.9 mV (Cs-based solution) were not corrected.

The following pharmacological agents were used: 6,7-dinitroquinoxaline-2,3-dione (DNQX), (±)2-amino-5-phos phono-pentanoic acid (D-APV), SR 95531, ZD7288, and tetrodotoxin (TTX) (all Tocris-Cookson, Bristol, UK), QX-314 and strychnine (Sigma-Aldrich, Deisenhofen, Germany). All drugs were dissolved in distilled water (10 mM), stored at −20 °C, and diluted in aCSF and washed in during the experiment.

Recording procedures

Slices were transferred to a recording chamber perfused (~3 ml per minute) with oxygenated aCSF at 36 ± 2 °C using an in-line solution heater (SH27B; Warner Instruments, Hamden, USA). Putative SPON principal cells were viewed with an upright microscope (Zeiss Axioscope, Oberkochen, Germany) equipped with a digital CCD camera (Orca 2, Hamamatsu, Tokyo, Japan) using a 40x-water-immersion objective (Achroplan, Zeiss) and infrared-differential interference optics. The SPON cells were visually identified by their large somata in a clearly delineated area medial to the LSO. Principal cells of the LSO were identified by their fusiform cell somata. Neuron size was estimated from the capacitance compensation measurement under voltage clamp. Only large neurons with a capacitance > 20 pF were included in the analysis. Whole-cell current-clamp recordings were performed throughout the SPON with a Multiclamp 700B amplifier (Axon Instruments, Foster City, CA, USA) using borosilicate glass microelectrodes with a final tip resistance of 3–10 MΩ. The bridge balance was continuously applied for current-clamp recordings. Evoked synaptic responses were elicited with a glass microelectrode filled with 2 M NaCl, which was positioned in the IAS