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Neuroproteomics in the auditory brainstem: Candidate proteins for ultrafast and precise information processing

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ABSTRACT

In the mammalian auditory brainstem, the cochlear nuclear complex (CN) and the superior olivary complex (SOC) feature structural and functional specializations for ultrafast (<1 ms) and precise information processing. Their proteome, the basis for structure and function, has been rarely analyzed so far. Here we identified and quantified the protein profiles of three major auditory brainstem regions of adult rats, the CN, the SOC, and the inferior colliculus (IC). The rest of the brain served as a reference. Via label-free quantitative mass spectrometry and 2-D DIGE/MALDI-MS, we identified 584 and 297 proteins in the plasma membrane/synaptic vesicle proteome and the cytosolic proteome, respectively. 'Region-typical' proteins, i.e., those with higher abundance in one region than in the other three, were considered candidates for functional specializations. Key proteins were validated via Western blots and immunohistochemistry. Functional annotation clustering revealed an overrepresentation of neurofilament proteins among the CN + SOC-typical proteins. These are related to regulation of axon diameter and, thereby, conduction velocity. Interestingly, the sets of synapse-associated proteins differed between regions. For example, synaptotagmin-2 (Syt2), a Ca^{2+} sensor for fast exocytosis, was CN + SOC + IC-typical, whereas Syt1 was CN + SOC + IC-atypical. Together, our quantitative comparison of protein profiles has revealed several interesting candidate proteins for ultrafast and precise information processing.

Introduction

Unraveling the molecular complexity of individual brain regions and revealing region-specific differences are fundamental challenges to comprehend brain function. Towards this goal, elucidation of the specific properties of the multitude of distinct brain regions by transcriptomic or proteomic approaches is required. As mRNA levels are relatively weak predictors of protein levels (de Godoy et al., 2008; Gygi et al., 1999), proteomics is usually preferred. Qualitative and quantitative catalogues of the neuroproteome can be generated via expression neuroproteomics (aka protein profiling), a powerful tool to uncover a high number of proteins (Bayes and Grant, 2009). Noticeably, more synaptic proteins have been discovered via proteomics than with any other method (Bayes and Grant, 2009; Takamori et al., 2006). The aim of the present comparative profiling study was to address differences and similarities in protein composition across various regions of the mammalian central auditory system (CAS).

The CAS comprises several serially connected regions (Fig. 1A). Five main relay stations are positioned between the auditory nerve and the primary auditory cortex, and most relay stations reside in the brainstem (Middlebrooks and Arbor, 2009). The cochlear nuclear complex (CN) in the medulla oblongata is the first CAS region from which second-order fibers emanate to innervate neurons in the superior olivary complex (SOC) and the inferior colliculus (IC) (Cant and Benson, 2003). The IC is an obligatory, mesencephalic hub from which efferent fibers emerge towards the medial geniculate body in the diencephalon (Middlebrooks and Arbor, 2009). Aside from being postsynaptic targets of CN fibers, IC neurons also receive input from SOC neurons. One hallmark of the lower brainstem centers in the CAS is precise temporal stimulus coding, which is essential to detect tiny inter-aural time differences in the microsecond range (Oertel, 1999). These tiny differences, in turn, are a cue to sound localization in the horizontal plane. Moreover, auditory brainstem neurons can fire action potentials at very high rates, up to 1000 per second (Golding and Oertel, 2012). Specific morphological, synaptic, and ion channel properties contribute to these remarkable and specific features (Fig. 1) (Borst and Sakmann, 1998; Golding and Oertel, 2012; Kramer et al., 2014; Oertel, 1999; Ryugo et al., 1998;

Abbreviations: CAS, central auditory system; CN, cochlear nuclear complex; GO, Gene Ontology; IC, inferior colliculus; IHC, immunohistochemistry; LSO, lateral superior olive; MNTB, medial nucleus of the trapezoid body; MSO, medial superior olive; PM + SV, plasma membrane/synaptic vesicle fraction; ProFaMs, protein family members; qMS, quantitative mass spectrometry; Rest, rest brain; ROI, regions of interest; SOC, superior olivary complex; SPN, superior paraolivary nucleus; VAS, ventral acoustic stria.

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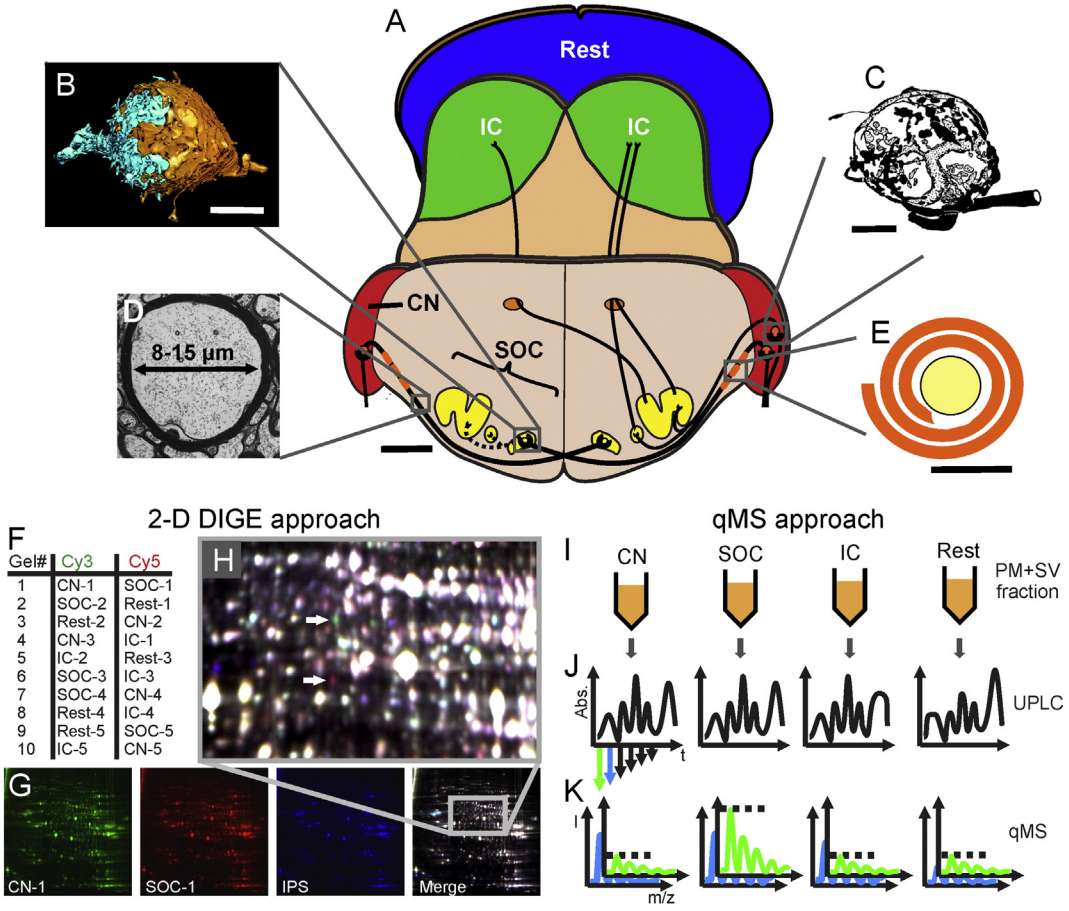

Fig. 1. Schematic illustrations of the four ROIs (CN, SOC, IC, Rest), structural specializations, and the two proteomic approaches. (A) Drawings of coronal sections through the rat brainstem, illustrating the CN (red), SOC (yellow), IC (green), and the rest of the brain (Rest, blue). The color coding is maintained throughout the paper. Axonal projections are also illustrated, and dotted line illustrates an inhibitory projection. (B–E) Specialized structures supporting ultrafast and precise information processing in the auditory brainstem: (B) calyx of Held (Sätzler et al., 2002), (C) endbulb of Held (Kretzmer et al., 2004), (D) large axon diameters (Paus and Toro, 2009), (E) myelinated axons: oligodendrocyte (orange) ensheathing an axon (yellow). Scale bars: 1 mm (A) and 10 µm (B, C, E). (F–H) 2-D DIGE approach. (F) Table illustrating the design of the 10 differential gels. Samples from ROIs were labeled with Cy3 or Cy5 as indicated. Thus, five biological replicates were obtained for each ROI (e.g. SOC-1 to SOC-5). (G) Representative 2-D DIGE gel images (CN-1, SOC-1, internal protein standard (IPS)), and a merge of all three channels. (H) Framed area in (G) at higher magnification. Asterisks show protein spots that are more intensive in CN-1 (green) or SOC-1 (red). (I–K) Quantitative mass spectrometry (qMS) approach. (I) Trypsinated PM + SV fractions of the four ROIs. (J) Samples were separated in time by nanoUPLC according to their hydrophobicity. (K) Separated peptides were analyzed on-line with a QTOF system run in MS^E acquisition mode (qMS); peptides were identified by their mass spectrum and quantified by the peak areas by PLGS. m/z = mass-to-charge ratio. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
Panel B is reprinted from Sätzler et al. (2002). Copyright 2002, with permission from the Society for Neuroscience. Panel C is reprinted from Ryugo et al. (1998). Copyright 1998, with permission from Wiley-Liss, Inc. Panel D is reprinted from Paus and Toro (2009). Copyright 2009, with permission from Paus and Toro.

Sätzler et al., 2002; Taschenberger and von Gersdorff, 2000; Trussell, 1999, 2008). Synaptic transmission in the CAS, particularly in the lower centers, is specialized for temporal precision and reliability, and both CN and SOC neurons display rapidly rising and falling postsynaptic potentials, thus preserving the timing of ultrafast synaptic inputs. Higher up in the brainstem (e.g. in the IC), auditory neurons have long membrane time constants and cannot phase lock to individual cycles of the stimulus (Ono and Oliver, 2014). Furthermore, they become more transient in their responses to sound (Frisina, 2001), and temporal processing occurs at a different time scale with lower temporal acuity. Information processing mainly includes amplitude modulation (Batra et al., 1993), gap detection (Walton et al., 1997), sound duration tuning (Aubie et al., 2009; Casseday et al., 1994; Pérez-González et al., 2006), and pre-pulse inhibition (Fendt et al., 2001), and these temporal features take place in the order of milliseconds to hundreds of milliseconds, much longer than in the CN and SOC. In line with this, the specific morphological, synaptic, and ion channel properties that are present in the CN and SOC (and associated with exquisite acuity) have not been detected in the IC. Synaptic transmission in the cerebral cortex is noisy (Smetters and Zador, 1996). Individual synapses are extremely unreliable and display a very low probability of transmitter release (Allen and Stevens, 1994), and the resulting input variability causes fluctuations in the timing and number of postsynaptic action potentials (Otmakhov et al., 1993).

We hypothesized that the performance at different time scales, as well as other typical properties, are mirrored by characteristic features in the protein profile of distinct CAS regions. To address this hypothesis, we performed a comparative profiling study in three CAS regions of interest (ROI): the CN, the SOC, and the IC. As a reference, we included the rest brain (Rest), to which the telencephalon, including the cerebral cortex, and the cerebellum contributed mostly.

The major aim of this study was the identification and validation of characteristic features in the protein profiles of the four ROIs, i.e., proteins that were significantly more abundant in one ROI compared with the three other ROIs. We refer to these proteins as region-typical proteins. Conversely, proteins that were significantly less abundant are considered region-atypical. To cover a broad range of proteins, we analyzed two subproteomes, namely that of the plasma membrane/synaptic vesicle fraction (PM + SV) and that of the cytosolic fraction. Towards this purpose, we performed label-free quantitative mass spectrometry and 2-D DIGE/MALDI-MS, respectively. In total, we identified 113 region-typical proteins. We confirmed and further analyzed key candidates, such as neurofilament proteins, synaptotagmin-2 (Syt2), and the potassium channel Kir4.1, using immunoblots and