L-Thyroxine improves vestibular compensation in a rat model of acute peripheral vestibulopathy

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28 **Abstract**

29 Unilateral vestibular lesions induce a vestibular syndrome, which recovers over time due to
 30 vestibular compensation. The therapeutic effect of L-Thyroxine (L-T4) on vestibular
 31 compensation was investigated by behavioral testing and immunohistochemical analysis in a
 32 rat model of unilateral vestibular neurectomy (UVN). We demonstrated that an acute L-T4
 33 treatment reduced the vestibular syndrome and significantly promoted vestibular
 34 compensation. Thyroid hormone receptors (TR α and TR β) and type II iodothyronine
 35 deiodinase (DIO2) were present in the vestibular nuclei (VN), supporting a local action of L-
 36 T4. We confirmed the T4-induced metabolic effects by demonstrating an increase in the
 37 number of cytochrome oxidase-labelled neurons in the VN three days after the lesion. L-T4
 38 treatment modulated glial reaction by decreasing both microglia and oligodendrocytes in the
 39 deafferented VN three days after UVN and increased cell proliferation. The survival of newly
 40 generated cells was not affected, but neuronal differentiation was altered by the L-T4
 41 treatment.

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Introduction

A unilateral vestibular lesion induces in most species including humans a characteristic vestibular syndrome composed of oculomotor, posturo-locomotor and perceptive-cognitive deficits. These symptoms recover over days to weeks due to a well-known phenomenon called vestibular compensation. In neuroscience, this functional recovery is considered as a good example of postlesional plasticity. Vestibular compensation is based on various plasticity mechanisms to restore a balanced activity between bilateral vestibular complexes in the brainstem (1–3).

Pharmacological modulation of the level of excitability or neurogliogenesis in the deafferented vestibular nuclei (VN) modifies the kinetics of vestibular functional restoration. Indeed, the pharmacological blocking of SK channels (small conductance calcium-activated potassium) by apamin, which inhibits the hyperpolarization phase of the action potential, facilitates the restoration of posturo-locomotor function and gaze stabilization (4). Among the numerous mechanisms of plasticity promoting vestibular compensation, we demonstrated that a unilateral vestibular neurectomy (UVN) induces a reactive neurogenesis in the deafferented vestibular nuclei of adult cats and rats (5–10). Continuous intracerebroventricular administration of an antimitotic drug immediately after UVN completely blocks the cellular proliferation and neurogliogenesis and delays the posturo-locomotor function recovery drastically (5). Conversely, increasing cell proliferation and survival with a continuous infusion of brain derived neurotrophic factor (BDNF) immediately after UVN accelerates the recovery of balance and posture while blocking BDNF-TrkB signalling significantly reduces cell proliferation and prevents postural locomotor recovery in animals with vestibular injuries (8). These data indicated that the priority of the deafferented vestibular environment after

UVN is to promote both the restoration of a level of homeostatic excitability between homologous VN and the expression of neurogliogenesis for vestibular functional recovery.

Interestingly, thyroid hormones (TH) can promote these two important mechanisms essential for vestibular functional recovery. TH signalling governs many aspects of neurogenesis, including proliferation, survival, migration, differentiation and maturation of neuronal and glial cells (11–14). Thyroxine (T4) or triiodothyronine (T3) injections have been shown to increase the expression of BDNF in different pathological models (stroke : (15, 16), Alzheimer disease: (17), and axotomy (18)). In addition, several studies have demonstrated the protective role of TH and their ability to promote functional recovery through genomic and non-genomic actions (for review see(19, 20)). Finally, even though the molecular events by which TH regulate energy metabolism are still not fully understood (21–23), this essential function could rebalance the activity between the two homologous VN and promote vestibular compensation. Based on all this evidence, TH are likely candidates to alleviate acute vestibular syndrome and facilitate vestibular compensation.

The aim of our study is to determine whether acute pharmacological treatment with L-thyroxine following UVN in adult rats affects the time course of vestibular functional recovery and the post-lesion plasticity mechanisms in the deafferented VN.

Results

L-Thyroxine treatment reduced postural vestibular deficits

After UVN, animals showed severe signs of posturo-locomotor alterations characteristic of vestibular syndrome, that can be scored using different tests. L-T4 (10 µg/kg, i.p.) or 0.9% NaCl (vehicle, in equivalent volume, i.p.) was administered to rats at the end of the UVN

procedure and a single injection per day over the first 3 days, 30 min before behavioral tests (Figure 1A).

These behavioral deficits were assessed at several time points over a period of 30 days using a qualitative ‘vestibular score’ scale (see Methods section). In both the UVN-NaCl and UVN-T4 groups, the mean vestibular score peaked on D1 (UVN-NaCl: 17 ± 0.53 ; UVN-T4: 12 ± 0.53) and never completely disappeared 30 days after the lesion (Figure 1B). From D1 until D7, animals treated with L-T4 significantly reduced the mean vestibular scores in the UVN-T4 group relative to the UVN-NaCl ($p < 0.001$) and then the UVN-T4 group reached similar values for the mean vestibular score to the UVN-NaCl group between D10 and D30 ($p > 0.05$).

Unilateral vestibular lesion also results in an increase in the support surface delimited by the animal’s four paws. This parameter is a good estimate of postural stability and restoration of balance (4, 8, 24, 25). When we compared the support surface area of each group between the preoperative values and the values obtained after UVN (D1-D30), we observed that the UVN-NaCl group significantly increased their support surface area the first 3 days following vestibular lesion (D1 to D3: $p < 0.001$) and reached pre-operative control values from D7 until D30 (Figure 1C). In contrast, the UVN-T4 group significantly increased their support surface area only at D1 after vestibular lesion (D1: $p < 0.001$) restoring to pre-operative control values from D2. When we compared the support surface area between the UVN-NaCl and UVN-T4 groups we observed that L-T4 treatment significantly reduced the support surface on D2, D3 and D10 compared to a saline injection after UVN (D2 and D3: $p < 0.001$; D10: $p < 0.01$).

To further study postural impairment after UVN and the improvement by L-T4 treatment, we next tested the weight distribution of rats on the lateral axis. As shown in previous reports (24, 26), before UVN, rats distributed their weight symmetrically between

the right and the left sides (**Figure 1D**). After UVN, UVN-NaCl rats increased the weight applied on their ipsilesional paws resulting in a postural asymmetry that reaches 8% at D7 and increases to 11% at D30 (D7: $p < 0.05$; D10 to D30: $p < 0.001$; **Figure 1D**). L-T4 treatment suppressed postural asymmetry induced by UVN from D7 to D30 (D7 to D30: $p < 0.05$). Taken together, these results suggest that L-T4 treatment improves the restoration of postural deficits induced by UVN.

L-Thyroxine treatment improved locomotor recovery after UVN

To investigate other behavioral alterations induced by L-T4 treatment after UVN, we conducted locomotor behavioral test batteries. We previously reported that UVN resulted in significant changes in locomotor activity (27). In open field test, L-T4-treated rats after UVN only significantly reduced their total distance travelled on D1 and L-T4 treatment prevented the hyperactivity from D10 to D30 observed in the UVN-NaCl group (**Figure 2A, B**). In contrast, the total distance travelled was remarkably diminished the first 3 days after UVN for the UVN-NaCl group (D1: $p < 0.001$; D2: $p < 0.01$; D3: $p < 0.05$) and conversely, increased from D7 until D30 (D7: $p < 0.05$; D10 to D30: $p < 0.001$). Similarly, immobility time of UVN-NaCl rats significantly increased the first 3 days after the lesion with a maximum on D1 (D1 to D3: $p < 0.001$) before returning to pre-operative control values from D7 (**Figure 2C**). Immobility time in L-T4-treated rats significantly increased only on D1 compared to pre-operative control values (D1: $p < 0.001$) and was significantly diminished on D1 and D2 after UVN compared to a saline injection (D1: $p < 0.001$; D2: $p < 0.05$). Mean velocity of UVN-NaCl group (**Figure 2D** and **Supplementary Video 1**) was significantly reduced the first 3 days after UVN (D1 and D2: $p < 0.001$; D3: $p < 0.01$) and significantly increased from D10 to D30 (D10 to D30: $p < 0.001$). L-T4 treatment prevented the reduction of velocity the first 3

days after UVN (**Supplementary Video 2**) but was significantly different from UVN-NaCl group only on D3 ($p < 0.05$). However, L-T4-treated animals also adopted the locomotor strategy of increasing their velocity from D10 to D30 (D10 to D21: $p < 0.05$; D30: $p < 0.01$). In the same way, the number of high accelerations (**Figure 2E**) of the UVN-NaCl group was almost absent the first 3 days (D1 to D3: $p < 0.001$) and significantly increased from D14 to D30 (D14: $p < 0.01$; D21 and D30: $p < 0.05$). L-T4-treated animals only significantly reduced their high accelerations on D1 and D2 (D1: $p < 0.001$; D2: $p < 0.05$) and increased significantly sooner (D7) their high accelerations compared to the UVN-NaCl group (D7: $p < 0.01$). The number of high accelerations of L-T4-treated animals was significantly different from UVN-NaCl rats on D3 and D7 (D3: $p < 0.05$; D7: $p < 0.01$). Finally, vestibular disorders induce imbalance and stumbling which leads to difficulty in walking straight. The ‘meander’ parameter from Ethovision XT14 allowed us to quantify this difficulty of walking (**Figure 2F**). While animals injected with a saline solution have a drastically unstable gait on D1 post-UVN (D1: $p < 0.001$), animals treated with L-T4 did not exhibit significant gait instability (D1: $p < 0.001$). Collectively, these findings suggest that an acute L-T4 treatment reduces the vestibular syndrome after a unilateral vestibular loss and promotes functional recovery (**Supplementary Video 1,2**).

Presence of thyroid hormone receptors and thyroxine deiodinase 2 in the vestibular nuclei

The results obtained from behavior analysis after UVN show that L-T4 treatment improved recovery of vestibular functions and suggest an effect of L-T4 in the vestibular nuclei (VN). The genomic effects of TH are mediated by the interaction of T3, the active form of TH, with TH receptors to act as transcriptional factors regulating the expression of several genes (21,

28). A first approach to understand the mechanism of action of TH in the VN consisted in analyzing the expression of TR α , TR β and iodothyronine deiodinase type 2 (DIO2) responsible for converting T4 into T3 (20, 29–31). We found that VN of adult rodent brain expressed both TR α and TR β and the enzyme DIO2 (**Figure 3A**). We also observed a negative correlation between TR α and SOX2 (**Figure 3B**) which is strikingly similar to the pattern reported in the subventricular niche (32). This result suggests a role of TH in cell proliferation and neuronal differentiation in the VN (**Figure 3B**). Overall, the presence of TH receptors and the DIO2 enzyme in the VN strongly suggest a local action of the L-T4 treatment.

L-Thyroxine increases cell proliferation and alters the cellular fate of newly generated cells in the deafferented vestibular nuclei after UVN

Unilateral vestibular loss induces numerous plastic events in the vestibular nuclei (3). Among these events are strong cell proliferation, glial reaction and reactive neurogenesis after UVN, which are all involved in vestibular compensation (5–8, 33). We recently demonstrated that quiescent neural stem cells in the MVN are reactivated after UVN (10) and interestingly TH play an important role in the proliferation and differentiation of neural stem cells (32, 34). Since TH affects glial cells function and governs many aspect of neurogenesis (11, 12, 31, 35), we investigated whether L-T4 treatment modulates reactive neurogenesis and glial reaction in the vestibular nuclei after UVN.

We evaluated cell proliferation with a single injection of BrdU (200 mg/kg) 3 h prior to intracardiac perfusion at D3 post-UVN (**Figure 4A**). L-T4 treatment significantly increased the mean number of BrdU⁺ cells in the deafferented MVN at D3 compared to the UVN-NaCl group (**Figure 4B,C**; $p < 0.001$). However, the number of proliferative cells (BrdU⁺)

decreased significantly in the UVN-T4 group at D30 reaching the values observed in the UVN-NaCl group.

Interestingly, TR α ⁺ cells increased significantly at D3 after UVN in animals treated with L-T4 compared to basal levels ($p < 0.001$; **Supplementary Figure 1A,B**). Conversely, the number of SOX2⁺ cells at D3 after UVN remains at the basal levels in the UVN-T4 group but increased significantly in the UVN-NaCl group ($p < 0.05$; **Supplementary Figure 1A,C**). The negative correlation between the number of SOX2⁺ cells and TR α ⁺ cells suggest that L-T4 treatment has an effect on the neural stem cells activated after UVN (10) by repressing the action of *Sox2* as described above in the subventricular neurogenic niche (32).

We then evaluated the cellular fate of the newly generated cells (BrdU⁺) with a single injection of BrdU (200 mg/kg) at D3 post-UVN prior to intracardiac perfusion at D30 (study design in **Figure 4A**). Cellular markers were revealed by double immunohistochemical labelling presented in **Figure 4D**. In the MVN of the UVN-NaCl group it was possible to determine the presence of newly generated neurons (BrdU⁺/NeuN⁺), astrocytes (BrdU⁺/GFAP⁺) and microglia (BrdU⁺/IBA1⁺) in almost equivalent proportion (~20%) with a predominance of the oligodendrocyte (BrdU⁺/Olig2⁺) phenotype (32%) (**Figure 4E**). Based on the literature, TH promote the engagement of neural stem cells preferentially towards a neural fate (14, 32, 34, 36). Surprisingly, with our paradigm, it seems that L-T4 treatment after UVN increased the formation of new microglial cells (41%) to the expense of new neurons (5%) (**Figure 4F**).

L-Thyroxine modulates the glial reaction and prevents downregulation of KCC2 in the vestibular nuclei after UVN

As reported in our previous studies, UVN induces a significant increase in microglia (IBA1), astrocytes (GFAP) and oligodendrocytes (Olig2) at D3 post-lesion in the deafferented vestibular nuclei (5–8, 10) (**Figure 5A-D**). L-T4 treatment significantly reduced the number of microglial cells ($p < 0.001$) compared to UVN-NaCl rats at D3 post-lesion (**Figure 5A,B**). However, the number of microglial cells in the UVN-T4 group was still significantly increased compared to preoperative values ($p < 0.001$). No difference in the number of astrocytes was found between the UVN-NaCl and UVN-T4 groups (**Figure 5A,C**). Finally, L-T4 treatment significantly reduced the number of oligodendrocytes at D3 compared to both the UVN-NaCl group ($p < 0.001$) and preoperative values ($p < 0.01$) (**Figure 5A,D**). At D30 post-lesion, the number of oligodendrocytes for the UVN-T4 group was comparable with the UVN-NaCl group (**Figure 5D**).

We previously assumed that UVN-induced microglia upregulation triggered a microglia-BDNF-TrkB signalling that decreased KCC2 expression in the deafferented vestibular nuclei (8). This hypothesis of a downregulation of KCC2 by BDNF and microglia is supported by other studies (37–39). As we showed a decrease in microglia with L-T4 treatment at D3, we wondered if KCC2 expression was also altered. While UVN-NaCl animals had reduced KCC2 expression at D3 post-lesion compared to pre-operative values ($p < 0.001$), L-T4 treatment restored KCC2 expression and no difference was found for the UVN-T4 group in comparison with pre-operative values (**Figure 5E,F**). Restoration of KCC2 expression was achieved one month after the lesion for the UVN-NaCl group. However, we observed a significant increase in KCC2 expression for UVN-NaCl rats compared to UVN-T4 rats 30 days after UVN ($p < 0.001$; **Figure 5F**).

L-T4-treated rats exhibit enhanced metabolic activity in the vestibular nuclei three days after UVN

It has been known for a century that thyroid hormones regulate energy metabolism (21, 22, 40). Quantification of cytochrome oxydase (CO) labelled neurons can provide insight into the metabolic effect of L-T4 in the vestibular nuclei after UVN. We focused on the lateral vestibular nucleus (LVN) in view of the extensive vestibulo-spinal projections of this nucleus implicated in gait and balance (41, 42) both at an acute (D3) and a compensated stage (D30) after UVN (summary of the study design presented in **Figure 6A**). L-T4 treatment induced a significant increase in the number of CO-labelled neurons on both ipsi- and contralesional LVN compared to both sides of the LVN in the UVN-NaCl group at 3 days post-UVN (**Figure 6B,C**; $p < 0.001$). One month after the lesion, both groups had a lower number of CO-labelled neurons and there was no significant difference between ipsi- or contralesional nuclei and between groups (**Figure 6B,D**). These data suggest that L-T4 treatment enhanced metabolic activity in the LVN the first days after the lesion but not at long term, which is consistent with the behavioral results.

Discussion

The effect of an acute L-T4 treatment on the vestibular syndrome following UVN is striking: all the vestibular behavioral parameters we analyzed showed a significant improvement. Our qualitative assessment of the vestibular syndrome indicated about 30% reduction in symptoms in the UVN-T4 group at the first day after the lesion. In addition, L-T4 treated animals reached the plateau of vestibular compensation earlier than untreated animals (between 3 and 7 days vs. 10 days). Based on our previous work (24, 26, 27), we analyzed more specifically the posturo-locomotor syndrome and demonstrated that L-T4 treatment promoted a faster recovery of postural and locomotor functions that had been impaired by UVN. The reported

improvements in walking speed and acceleration in L-T4 treated rats can be directly transposed to the clinic. Increasing walking speed for vestibular patients is considered as an improvement during a vestibular rehabilitation program (43–45). Furthermore, changes in gait speed and accelerations can be used to assess dynamic postural stability (e.g., by Dynamic Gait Index) (46) and can be used in vestibulospinal exercises for vestibular rehabilitation (45, 47, 48). The future clinical implications of the beneficial effect of TH on vestibular compensation for therapy of patients with acute vestibular loss can be two-fold: 1) Similarly to the current rat experiments, L-T4 could be administered in the acute phase of vestibular syndrome to reduce symptoms of vestibular asymmetry and accelerate vestibular compensation. The translation to clinical practice is facilitated by the widespread availability of L-T4 medication. 2) Pre-existing hypothyroidism may be considered as a risk factor for poor vestibular compensation or vertigo, which should be screened more regularly (49–51).

Despite the fascinating physiological effect of L-T4 on vestibular function recovery in UVN rats, the intimate mechanisms by which thyroid hormones act remain to be elucidated. However, the different symptoms observed after unilateral vestibular loss result from asymmetry of spontaneous electrical activity between homologous VN (52, 53). The data in the literature show that the restoration of electrophysiological equilibrium between the two opposite VN is the key parameter for the functional recovery of postural, locomotor and gaze stabilization functions (2, 54, 55). TH are known to stimulate diverse metabolic activities resulting in an increase in ATP and oxygen consumption (22, 56, 57). In line with the literature, the L-T4-treated group exhibited a bilateral increase in the number of CO-labelled neurons in the LVN three days after UVN compared to the UVN-untreated group. Moreover, the increased number of CO labelled neurons observed in the deafferented LVN in the UVN-T4 group was significantly higher than that observed in the contralesional LVN of the UVN-untreated group, highlighting a drastic increase in metabolic activity and ATP production due

to L-T4 treatment. As Mori et al. demonstrated, intravenous administration of ATP produces an increase in spontaneous neuronal discharge of MVN and LVN neurons (58). Thus, stimulation of ATP production by L-T4 treatment could increase the excitability of ipsilesional vestibular neurons reducing electrophysiological asymmetry between homologous vestibular nuclei leading to an attenuation of the syndrome and an acceleration of vestibular compensation.

Interestingly, the deafferented vestibular environment has been reconfigured to ensure the binding of endogenous and exogenous thyroid hormones by increasing TR α expression in the UVN-T4 group (**Supplementary Figure 1**). Furthermore, we can assume that the astroglial reaction observed in UVN animals actively participates in the conversion of L-T4 in T3 and supports all the mechanisms promoting vestibular compensation in response to the metabolic energy demand of the deafferented VN. Indeed, a proteomic analysis in the MVN of rats one week after unilateral labyrinthectomy (UL) showed an increase in proteins associated with mitochondrial function and energy metabolism (59). One of these proteins was succinate dehydrogenase, a crucial enzyme in the mitochondrial Krebs cycle, for which positive regulation was observed at 5 and 15 days after UVN in the ipsilesional LVN of rabbits (60). Overall, these changes reflect the high metabolic energy demand of the deafferented VN associated with glial response, cell proliferation, neurogenesis, and structural remodeling to compensate for the loss of vestibular input.

Based on other studies (37–39), we have proposed that UVN-induced upregulation of microglia triggers a microglia-BDNF-TrkB signalling pathway that decreased KCC2 expression in the deafferented VN (8). In the present study, we demonstrate that L-T4 treatment prevents the negative regulation of KCC2 in the deafferented LVN compared to the UVN-NaCl group. However, an opposite action of BDNF on KCC2 expression has been described in axotomized corticospinal neurons (61). Whereas in intact mature neurons, BDNF

downregulated KCC2 (39, 62, 63), in injured neurons BDNF upregulated KCC2 (8, 61, 62). Interestingly, TH have been shown to increase the expression of BDNF in lesioned slices of the hippocampus (18, 64) and other pathological models (15–17). We can then assume that an acute L-T4 treatment after UVN induces a local increase of BDNF in the deafferented VN, thus promoting cell proliferation as demonstrated in our previous study (8). Finally, the absence of KCC2 downregulation induced after L-T4 treatment is consistent with the hypothesis of Kaila et al., which proposes that “downregulation of KCC2 following neuronal trauma may be part of a general adaptive cellular response that facilitates neuronal survival by reducing the energetic costs that are needed to preserve low $[Cl^-]_i$ ” (65). By providing TH, known to increase energy metabolism, the internalization of KCC2 to reduce energy costs after UVN would no longer be necessary.

Unsurprisingly, L-T4 injections after UVN reduce the number of Olig2⁺ cells and prevent the increase of SOX2⁺ cells in the deafferented VN. These results are congruent with the literature (32, 66) and are in agreement with the increased cell proliferation observed in the UVN-T4 group three days after the lesion. However, in our paradigm we did not observe cell differentiation preferentially towards a neuronal fate (BrdU⁺/NeuN⁺ cells) with the L-T4 acute treatment. On the contrary, cell differentiation is promoted towards a microglial cell phenotype (BrdU⁺/IBA1⁺ cells) in the UVN-T4 group. This result could appear paradoxical since TH promote the engagement of neural stem cells preferentially towards a neuronal fate (14, 32, 34, 36). Yet our results do not contradict this well-established concept. As expected, L-T4 injections during the first three days post-UVN increased cell proliferation on the third day. However, cell differentiation was evaluated at day 30 which is 4 weeks after the last L-T4 injection. Given the thyroxine half-life of 12-24 hours in rats (67), the treatment is eliminated from the system when cell proliferation is assessed. We believe that an extended treatment window would have revealed an increase in neuronal differentiation as described in

the literature. Another explanation could come from the reduction of the vestibular syndrome observed with L-T4 treatment in the first days after injury. Neurogenesis is indeed expressed in the UVN model but not in the labyrinthine lesion or hair cell destruction model (6). What differentiates these three models is the severity of the vestibular syndrome: most severe in the UVN model and weaker in the other two models. Since the UVN-vestibular syndrome is considerably reduced by L-T4 treatment and the vestibular compensation is accelerated (more akin to the kinetics of a labyrinthine lesion model as discussed above), it is possible that neuronal differentiation is not necessary to compensate for the deficits. In agreement with this hypothesis, we have demonstrated a decrease in the percentage of BrdU⁺/NeuN⁺ in cats treated with BDNF showing an improvement in vestibular compensation (8). With this hormonal treatment we provide other compensation pathways to the central nervous system, notably based on energy metabolism and excitability, which could explain the limited number of newly generated neurons we observed in our model.

Obviously, the results obtained support a local action of TH in the deafferented VN. However, given the intraperitoneal administration we cannot exclude an effect on the whole central network of vestibular compensation (68, 69) and peripheral effect. Skeletal muscle is in fact a primary target for TH signalling in human and rodents (70). TH are involved in contractile function by increasing the rate of muscle relaxation-contraction, in energy metabolism by increasing mitochondrial biogenesis, but also in myogenesis and muscle regeneration (70, 71). Interestingly, UVN induces asymmetry of muscle tone with ipsilesional hypotonia and contralesional hypertonia (72, 73), thus altering the postural and locomotor balancing function. While no studies to date have demonstrated an increase in muscle tone after TH administration, it is plausible to assume that by increasing contractile function and muscle metabolism, L-T4 treatment could accelerate the rebalancing of muscle tone after UVN, which is crucial for postural stability.

In summary, acute administration of L-T4 after unilateral vestibular loss greatly reduces vestibular syndrome and improves vestibular compensation. The present results demonstrate different mechanisms of action of L-T4 underlying the antivertigo effect and open new perspectives of L-T4 treatment for acute vestibular loss.

Methods

Animals

The experiments were performed on 24 adult Long Evans rats (250/300g) originating from our own breeding, from parents obtained from Charles River (St Germain sur l'Arbresle, France). All experiments were performed in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH Publication no. 80-23) revised in 1996 for the UK Animals (Scientific Procedures) Act of 1986 and associated guidelines or the Policy on Ethics approved by the Society for Neuroscience in November 1989 and amended in November 1993 and under the veterinary supervision and control of the National Ethical Committee (French Agriculture Ministry Authorization: B13-055-25). The present study was specifically approved by Neurosciences Ethic Committee N°71 of the French National Committee of animal experimentation. Every attempt was made to minimize both the number and the suffering of animals used in this experiment. The animals were housed in a large confined space with 12h-12h diurnal light variations with free access to food and water.

Unilateral vestibular neurectomy

Animals were subjected to a left-side vestibular nerve section (n=24) following the surgical procedure previously reported in the literature (Péricat et al., 2017). 30 min after a subcutaneous injection of buprenorphine (Buprecare®; 0.02 mg/kg), the rats were placed in the induction box and left for 5 min (isoflurane concentration 4%). Once they were deeply anesthetized, they were intubated and, during the surgery, the anesthesia was maintained at an isoflurane concentration of 3%. A tympanic bulla approach gave access to the vestibular nerve: the cervical muscular planes were dissected leading to the tympanic bulla, which was widely drilled to expose the stapedial artery and the promontory containing the cochlea. The cochlea was drilled, exposing the cochlear nerve. The cochlear nerve meatus was enlarged with a needle leading to the vestibulocochlear nerve, which was sectioned at its entry into the brainstem after aspiration of Scarpa's ganglion. The wound was closed using a stapler. Before awakening, the animals received either an intraperitoneal injection of 0.9 % NaCl (UVN-NaCl group) or a solution of thyroxine (UVN-T4 group, 10 µg/kg). A solution of Ringer Lactate (Virbac; 10 ml/kg) was also administered subcutaneously in order to alleviate the dehydration resulting from the inability of the animal to drink normally as a result of the injury.

The success of the surgery was attested at the behavioral level by the presence of a characteristic vestibular syndrome and at the histological level by the observation of the full section of the 8th cranial nerve between Scarpa's ganglion and VN in the brainstem under optical microscopy (see Péricat et al., 2017 for details).

Criteria for Exclusion

Animals were excluded from the study if the following symptoms were observed:

- loss of body weight equal to more than 20% of the preoperative value.

- if the facial nerve had been sectioned.

- abnormalities in behavioral scoring, i.e., inability of the animal to stand on all four paws after 5 days post-UVN, convulsions, hemiataxia, etc.

Based on these criteria, one animal of the UVN-T4 group was excluded.

Study design

To determine the effects of thyroxine injection after UVN on the different steps of reactive neurogenesis at the cellular level and on the time course of vestibular functional recovery at a behavioral level, animals were divided into 4 groups as follows: (1) rats underwent UVN and received intraperitoneally one NaCl injection at the end of the surgery and one injection/day during the first 3 days post-lesion (UVN-NaCl group, n=12). These animals received a BrdU injection (200 mg/kg, i.p.) on day 3 post-UVN and were killed at either day 3 when cell proliferation reached a peak (Group 1, n=4) or day 30 post-lesion to study vestibular compensation with the survival and the differentiation of the proliferating cells (Group 2, n=8). (2) rats underwent UVN and received intraperitoneally one L-thyroxine (L-T4) injection (10 µg/kg, dissolved in saline, Sigma, Cat#T2501) at the end of the surgery and one injection/day during the first 3 days post-lesion (UVN-T4 group, n=12). These animals received a BrdU injection (200 mg/kg, i.p.) on day 3 post-UVN and were killed at either day 3 (Group 3, n=4) or day 30 post-lesion (Group 4, n=8).

Group 2 (NaCl, n=8) and Group 4 (T4, n=8) underwent behavioral investigation prior to UVN and on day 1, 2, 3, 7, 10, 14, 21 and 30 post-lesion to evaluate the time course of vestibular functional recovery before they were killed for cellular investigation. In order to establish the full time course of the vestibular compensation after UVN, the data from the present study were combined with other data recorded in a previous work (27). We

administered L-T4 (10 µg/kg, i.p.) or 0.9% NaCl (vehicle, in equivalent volume, i.p.) during the first 3 days to rats 30 min before behavioral tests.

Qualitative assessment of the vestibular syndrome

We assessed qualitatively the vestibular syndrome following UVN. Behavioral symptoms of vestibular imbalance were scored for 10 components after UVN: the tail-hang test (adapted from (74–76)), rearing, grooming, displacement, head-tilt, barrel rolling, retropulsion, circling and bobbing. The tail-hang test quantifies typical vestibulo-spinal reflexes.

- Tail hanging behavior: Animals were picked up from the ground at the base of the tail and body rotation was scored from 0 point (no rotation) to 3 points (several rotations of 360°)

- Landing reflex: After animals were picked up from the ground at the base of the tail, we scored the first 3 landings from 0 (presence of a landing reflex on the 3 landings) to 3 points (absence of landing reflex on the 3 landings). When lifted by the tail, normal rats exhibit a landing reflex consisting of forelimb extension that allows them to land successfully (i.e. they land on all four legs). Rats with impaired vestibular function do not exhibit a forelimb extension, spin or bend ventrally, sometimes “crawling” up toward their tails, causing them to miss their landings.

- Rearing: the ability of the rat to rear was scored from 0 point (rearing is observed) to 1 point (rearing is absent)

- Grooming: the ability of the rat to groom correctly were scored as follows: 0 point (correct grooming of full body) 1 point (grooming of the face, belly and flanks but not the base of the tail), 2 points (grooming of the face and belly), 3 points (grooming of the face), 4 points (inability of the animal to groom itself)

-Displacement: quality of the displacement of the rat was scored from 0 (displacement of the rat with no visible deficit) to 3 points (several deficits in the displacement of the rat)

-Head tilt was scored by estimating the angle between the jaw plane and the horizontal with 0 points (absence of a head-tilt) to 3 points (for a 90° angle)

-Barrel rolling was scored as follows: 0 points (absence of barrel rolling), 1 point (barrel rolling evoked by an acceleration in the vertical axis of the rat in our hand), 2 points (spontaneous barrel rolling)

-Retropulsion characterizes backwards movements and was scored from 0 (absence of retropulsion) to 1 point (presence of retropulsion)

-Circling was scored from 0 point (absence of circling behavior) to 1 point (presence of circling behavior)

-Bobbing is assimilated to cephalic nystagmus and was scored from 0 point (absence of bobbing) to 1 point (presence of bobbing)

A first acquisition was done the day before the lesion, serving as a reference value, and then acquisitions were performed at days (D) 1, 2, 3, 7, 10, 14, 21 and 30 post-lesion.

Weight distribution

To quantify the postural syndrome following UVN we used a device (DWB1®, Bioseb, Vitrolles, France) measuring the weight distribution at all contact points of the animal's body with the ground. This apparatus has previously been described for the assessment of postural instabilities in the same model of vestibular loss (26). The device consists of a Plexiglas cage (25x25 cm) in which the animal can move freely. The floor of this cage is fully covered with a plate with 2000 force sensors. Sensors detect vertical pressure at a sample rate of 30 Hertz.

The sensors are connected to an electronic interface that converts the current flowing through it into a measure of weight, the whole being connected to a computer. The cage is closed by a lid on which is attached a high definition camera, also connected to the computer through a USB cable.

The analyses enabled the acquisition of a range of parameters calculated automatically by the software (for details, see Tighilet et al., 2017). Based on previous results (26), we chose to analyze only the weight distributed on the lateral axis, which allows determination of how the animal distributes its weight between the left and right paws in order to maintain balance.

For each acquisition session, the rat was placed in the device for 5 min and could move freely. The pre-operative session was recorded the day before the surgery, and then the time course of the vestibular syndrome was studied on days (D) 1, 2, 3, 7, 10, 14, 21 and 30 post-lesion.

Open field test

To quantify the locomotor syndrome following UVN we used an automated video tracking software (EthoVision™ XT 14, Noldus, Netherlands). Animals were individually placed in a square open field (80×80×40 cm) and could move freely for 10 min. Their behavior was recorded for 10 min using a digital camera and analyzed with EthoVision™ XT 14 software. Position of nose, body center and tail were automatically detected by video software.

Based on previous results (27), the mean distance travelled, meander, mean locomotor velocity, mean number of high accelerations ($>50 \text{ cm/s}^2$) and mean percentage of time immobile were quantified (for details, see (27)). To minimize stress, the room was lit as dimly as possible while allowing us to clearly discern the rats. At the beginning of the session, the rat was placed on the right side of the field, head facing the wall. A first acquisition

was done the day before the lesion, serving as a reference value, and then acquisitions were performed at days (D) 1, 2, 3, 7, 10, 14, 21 and 30 post-lesion.

Support Surface

Static postural deficits and recovery were evaluated by measuring the support surface delimited by the four legs of the rat after the tail hanging landing test. This test consisted in taking the animal by the tail and lifting it vertically to a height of about 50 cm (lift duration 2 s; position holding at upper position: 1 s). The support surface can be regarded as a good estimate of postural control because it reflects the behavioral adaptation of the rat compensating the static vestibulospinal deficits induced by the vestibular lesion. To quantify the support surface, rats were placed in a device with a graduated transparent floor that allowed them to be filmed from underneath. A scale drawn on the bottom served to take measurements of the location of the four paws. When the animal landed after the tail hanging landing test and touched the ground, we took a capture of the location of the four paws. Twenty repeated measurements were taken for each rat tested at each postoperative time, and an average was calculated for each experimental session. A first acquisition was made the day before the lesion, serving as a reference value, and then acquisitions were performed at days (D) 1, 2, 3, 7, 10, 14, 21 and 30 post-lesion. The support surface was measured using a custom written image analysis tool (Matlab®, Mathworks, Inc.). An average was calculated for each post-lesion time. Data recorded after UVN were compared to the mean pre-lesion values using individual references, which means each animal acted as its own control.

Tissue preparation

The rats were deeply anesthetized with a mixture of ketamine 1000/medetomidine and then perfused by intracardiac injection. The intracardiac injection of 400 ml of isotonic saline (0.9% NaCl) was followed by an infusion of 400 ml of freshly prepared Zamboni solution (4% paraformaldehyde and in 0.1 M phosphate buffer (PB), pH 7.4). At the end of the perfusion, the brain was extracted from the skull and post fixed overnight at 4 °C in the same fixative mixture as that used during the perfusion. Brains were rinsed and cryoprotected by successive transfers into increasing concentrations (10%, 20%, 30% of sucrose solution in 0.1M PB for 72 h at 4 °C). Brains were rapidly frozen with CO₂ gas and cut into serial 40 µm frontal section with a cryostat (Leica) for immunochemistry.

Immunohistochemistry

Immunochemical labelling was performed according to previously validated protocols (7, 8, 10). Cell proliferation was analyzed in groups 1 (UVN-NaCl-D3) and 3 (UVN-T4-D3), after injection of BrdU on D3 post-UVN and the animals were killed 3 h later. Survival and differentiation of the newly generated cells were analyzed in groups 2 (UVN-NaCl) and 4 (UVN-T4) that were injected with BrdU on D3 post-UVN and killed on D30. Floating brain sections were washed (3x10 min) with PBS in multiwell plates. Blocking was done by incubation (1 hour) in 5% BSA and 0.3% Triton X-100. Slides were incubated overnight at 4°C with the following primary antibodies: mouse anti-BrdU (1:100, Dako), rat anti-BrdU (1:100 Oxford Biotech), mouse anti-NeuN (1:100, Millipore), rabbit anti-GFAP (1:200, Dako), rabbit anti-IBA1 (1:2000, Wako), rabbit anti-Olig2 (1:500, Millipore), goat anti-Sox2 (1:100, R&D Systems), rabbit anti-TRα (1:200, Rockland), rabbit anti-TRβ (1:200, Invitrogen), goat anti-DIO2 (1:200, Invitrogen), and rabbit anti-KCC2 (1:200, Millipore). Fluorescent secondary antibodies (Abcam) were used as follows: Alexa Fluor 594 nm goat

anti-rat (1:500), Alexa Fluor 594 nm donkey anti-goat (1:500), Alexa Fluor 488 nm donkey anti-rabbit (1:500) and Alexa Fluor 488 nm donkey anti-mouse (1:500) for 2 hours at room temperature. Finally, brain sections were mounted onto SuperFrost/Plus glass slides (Fischer) and air-dried before being mounted with Roti®Mount FluorCare antifade reagent with the nuclear marker DAPI (Carl Roth).

Cells count and statistical analysis

For quantification of cells expressing specific markers, 1 in 12 serial sections starting at the beginning of the vestibular nuclei (relative to bregma, -9.84 mm) to the end of the vestibular nuclei (relative to bregma, -13.08 mm), according to the rat brain stereotaxic atlas (77), were used. Only sections of the medial vestibular nucleus (MVN) on the lesioned (left) side were evaluated. Quantification of BrdU-, NeuN-, GFAP-, IBA1-, Olig2-, Sox2-, thyroid hormone receptor alpha- ($TR\alpha$) and thyroid hormone receptor beta- ($TR\beta$) positive cells was counted using confocal imaging with a Zeiss LM 710 NLO laser scanning microscope equipped with a 63x/1.32 NA oil immersion lens. For each marker, immunoreactive positive cells in the MVN were counted using an integrated microscopic counting chamber that delineated the region of interest by a square of $425.10 \mu m^2$. The average cell counts from the sections were used for statistical analysis.

Quantification of KCC2

A custom program written in Matlab® (Mathworks, Inc) was developed to analyze the membrane fluorescence of KCC2 (78). The background or non-specific immunofluorescence was evaluated by calculating the mean fluorescence in a selected area devoid of neurons or

other immunolabelled structures. In this region we then established a threshold equal to the mean immunofluorescence plus three times the standard deviation. All data were subtracted from this threshold value and only positive values were conserved for further analysis. A region of interest was then drawn around the plasma membrane of a neuron. The program calculated the mean membrane fluorescence of each cell body in the region of interest over data that were 20% above the maximum values. This threshold ensured that all pixels taken for averaging were part of the plasma membrane, otherwise the average fluorescence may depend on how large the region of interest was manually drawn around the plasma membrane. The mean membrane fluorescence of the measured neurons enables us to estimate the level of membrane expression of KCC2. A low membrane expression of KCC2 is correlated with an increase in intracellular Cl^- ion concentration. The consequence of this increase is a decrease in the efficacy of GABAergic inhibition and, in the most severe cases, an exit of Cl^- ions from the cell by the action of GABA on its GABA_A receptor, thus converting GABAergic neurotransmission from inhibitory to excitatory (65).

Cytochrome oxidase histochemistry

Metabolic activity in the lateral vestibular nuclei was assessed by means of cytochrome oxidase (CO) labelling on series of 40 μm -thick coronal frozen sections. Slides were incubated overnight in the dark in a solution of 0.1 M phosphate buffer (pH 7.4) containing 10 mg of DAB (Tablet #D5905 Sigma-Aldrich, France) and 30 mg horse-heart cytochrome c (#C2506, Sigma-Aldrich, France) per 50 mL, stirred continuously (Sigma-Aldrich, France for all the chemical products). The slides were then washed two times in buffer (2x 5 min), dehydrated in successive ethanol and xylene baths, and coverslipped with DPX. Images of the lateral vestibular nuclei, identified by the rat brain stereotaxic atlas (77), were collected and

we counted the number of neurons positive to CO labelling only in the lateral vestibular nuclei.

Statistical Analysis

We performed all statistical analyses using GraphPad Prism software (version 7, GraphPad Software). Summary graphs were all shown as mean $\pm$ SEM. Two-way repeated measures ANOVA with post-hoc Bonferroni's multiple comparison test were used to determine statistical differences between the treatments (UVN-NaCl and UVN-T4) for behavioral data. The statistical analyses of the cellular data were evaluated by one-way ANOVA to determine the effects of the group (UVN-NaCl and UVN-T4) and the postoperative time (Pre-op, D3 or D30) on BrdU⁺, NeuN⁺, GFAP⁺, IBA1⁺, Olig2⁺, Sox2⁺, TR α ⁺, TR β ⁺, CO⁺ cells to determine whether there were any interactions between these variables. ANOVA was followed by post hoc analysis with the Tuckey's test. P value < 0.05 was considered as statistically significant. *p < 0.05, **p < 0.01, ***p < 0.001.

Author Contributions

G.R. and B.T conceived the study. G.R. performed behavioral pharmacology. G.R., I.W. and A.L. performed immunohistochemical experiments. D.P. developed and performed the surgeries. A.T provided expertise and contributed to confocal microscopy. G.R., E.M., N.E.M., A.L-J. and B.T. contributed to data analysis and interpretation of the results. P.C., M.J-F. and A.Z. provided medical expertise. G.R. and B.T. wrote the manuscript, and all authors contributed to its editing.

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Competing Interests statement

The authors declare no competing financial interests.

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Figure Legends

Figure 1: L-T4 treatment accelerates recovery from UVN-induced postural alterations.

(A) Study design used to assess the behavioral effects of acute L-thyroxine (L-T4) treatment after unilateral vestibular neurectomy (UVN). UVN-T4 group received L-T4 injections (10 µg/kg dissolved in saline, i.p.) and UVN-NaCl group received 0.9% NaCl (vehicle, in equivalent volume, i.p.) at the end of the UVN and one injection/day during the first 3 days post-lesion. (B) Curves illustrating the time course (on the abscissae) of the vestibular syndrome (vestibular score on the ordinates) of UVN-NaCl (red) and UVN-T4 (blue) groups. (C) Curves indicating the mean postoperative recovery of the support surface of UVN-NaCl and UVN-T4 groups. (D) Histograms illustrating the weight distribution on the lateral axis of UVN-NaCl and UVN-T4 groups. Error bars represent SEM. A significant difference from the pre-operative value is indicated by * in red for UVN-NaCl group. A significant difference from the pre-operative value is indicated by * in blue for UVN-T4 group. A significant difference between UVN-NaCl and UVN-T4 group is indicated with * in black (*p < 0.05, **p < 0.01, ***p < 0.001; repeated measure two-way ANOVA and Bonferroni's post tests).

Figure 2: L-T4 treatment accelerates recovery from UVN-induced locomotor alterations. (A) Curve illustrating the mean postoperative recovery of the total distance moved by UVN-NaCl and UVN-T4 rats in the open field. (B) Video-tracked path of a UVN-NaCl and a UVN-T4 rat one day after UVN in the open field over a 10 min period. (C) Curves illustrating the kinetics of the % of time when the animal is immobile of UVN-NaCl (red) and UVN-T4 (blue) groups. (D) Curves illustrating the kinetics of the mean velocity (cm/s) of the animals (calculated from the center-point) of UVN-NaCl and UVN-T4 groups. (E) Curves illustrating the kinetics of the mean number of high accelerations (above 50 cm/s²) of the animals (calculated from the center point) of UVN-NaCl and UVN-T4 groups. (F) Curves illustrating the mean postoperative recovery of meander (sinuous trajectory) of UVN-NaCl and UVN-T4 groups. Error bars represent SEM. A significant difference from the pre-operative value is indicated by * in red for UVN-NaCl group. A significant difference from the pre-operative value is indicated by * in blue for UVN-T4 group. A significant difference between UVN-NaCl and UVN-T4 group is indicated with * in black (*p < 0.05, **p < 0.01, ***p < 0.001; repeated measure two-way ANOVA and Bonferroni's post tests).

862 **Figure 3: Presence of TR α , TR β and Dio2 in the vestibular nuclei of control rats. (A)**
 863 confocal immunostaining images confirming the presence of TR α (green), TR β (green) and
 864 Dio2 (red) in the vestibular nuclei of control animals (without UVN). **(B)** Colocalization of
 865 TR α (green), SOX2 (red) and DAPI (blue) of cells in the vestibular nuclei. Head arrows
 866 indicate TR α ⁺/SOX2⁺/DAPI⁺ cells, arrows with tail indicate TR α ⁺/SOX2⁻/DAPI⁺ cells and
 867 little arrows with tail indicate TR α ⁻/SOX2⁺/DAPI⁺ cells. Scale bar = 20 μ m.

868

A

B

Figure 4: L-T4 treatment modulates cell proliferation and neurogenesis after UVN. (A)

Study design used to assess cell proliferation and survival of the newly generated cells after unilateral vestibular neurectomy (UVN) and L-thyroxine (L-T4) treatment. UVN-T4 group received L-T4 injections (10 µg/kg dissolved in saline, i.p.) and UVN-NaCl group received 0.9% NaCl (vehicle, in equivalent volume, i.p.) at the end of the UVN and one injection/day during the first 3 days post-lesion. At day 3, animals received a BrdU injection (200 mg/kg, i.p.) and were perfused either 3 hours later to evaluate cell proliferation, or on day 30 to investigate survival and differentiation of the newly generated cells. **(B)** Confocal immunostaining images of BrdU+ cells in the deafferented medial vestibular nucleus (MVN) before the lesion (pre-op), three days (D3) and thirty days (D30) after UVN for UVN-NaCl and UVN-T4 groups. Scale bar = 25µm **(C)** Quantitative assessment of the effect of UVN and L-T4 treatment on the number of BrdU+ cells in the deafferented MVN of UVN-NaCl (red) and UVN-T4 (blue) groups. (***) $p < 0.001$; One-way ANOVA and Tuckey's post tests). **(D)** Maximum intensity projection of Z-stack confocal images of cell differentiation evaluated in the deafferented MVN 30 days after UVN. The BrdU nuclei are in red and the other markers of differentiation: GFAP (astrocyte), IBA1 (microglia), Olig2 (oligodendrocytes) and NeuN (neuron) are in green. Scale bar = 10µm. **(E,F)** Proportions of new neurons (green), astrocyte (blue), microglia (orange), and oligodendrocytes (purple) generated in the deafferented MVN of the UVN-NaCl **(E)** and UVN-T4 **(F)** groups 30 days after the lesion.

Figure 5: L-T4 treatment modulates glial reaction in the deafferented medial vestibular nucleus after UVN. (A) Confocal immunostaining images of IBA1⁺, GFAP⁺ and Olig2⁺ cells in the deafferented medial vestibular nucleus (MVN) before the lesion (pre-op), three days (D3) and thirty days (D30) after unilateral vestibular neurectomy (UVN) for UVN-NaCl and UVN-T4 groups. Scale bar = 50µm. (B-D) Quantitative assessment of the effect of UVN and L-T4 treatment on the number of IBA1⁺ (B), GFAP⁺ (C) and Olig2⁺ (D) cells in the deafferented MVN of UVN-NaCl (red) and UVN-T4 (blue) groups. (E) Confocal immunostaining images in the deafferented lateral vestibular nucleus showing the KCC2 staining (green) in vestibular neurons of UVN-NaCl and UVN-T4 groups observed before the lesion (pre-op), D3 and D30 after UVN. (F) Quantification of the density of membrane labelling in vestibular neurons of intact rats (pre-op, n=37) and in UVN-NaCl (red) and UVN-T4 (blue) groups at either D3 (NaCl, n=36; T4, n=111) or D30 (NaCl, n=48; T4, n=113) after UVN. Scale bar = 20µm. Error bars represent SEM. A significant difference from the pre-operative value is indicated by * in red for UVN-NaCl group. A significant difference from the pre-operative value is indicated by * in blue for UVN-T4 group. A significant difference between UVN-NaCl and UVN-T4 group is indicated with * in black (*p < 0.05, **p < 0.01, ***p < 0.001; One-way ANOVA and Tuckey's post tests).

A

B

C

D

E

F

Figure 6: L-T4 treatment after UVN enhance metabolic activity in the vestibular nuclei.

(A) Study design used to assess cellular consequences of acute L-thyroxine (L-T4) treatment after unilateral vestibular neurectomy (UVN). UVN-T4 group received L-T4 injections (10 µg/kg dissolved in saline, i.p.) and UVN-NaCl group received 0.9% NaCl (vehicle, in equivalent volume, i.p.) at the end of the UVN and one injection/day during the first 3 days post-lesion. Animals were perfused either at an acute stage of vestibular compensation (3 hours after the BrdU injection on day 3) or at a compensated stage (on day 30). (B) Illustrations of cytochrome oxidase (CO) enzymatic labelling in the lateral vestibular nucleus (LVN) of UVN-NaCl and UVN-T4 group observed at three days (D3) and thirty days (D30) after unilateral vestibular neurectomy (UVN). Illustrations are shown on both contralesional and ipsilesional (deafferented) side. (C) Quantitative assessment of the effect of UVN and L-T4 treatment on the number of CO-labelled neurons in the LVN. Dashed line represents the pre-operative value of the number of CO-labelled neurons in the LVN. Error bars represent SEM. A significant difference from the pre-operative value is indicated by * in red for UVN-NaCl group. A significant difference from the pre-operative value is indicated by * in blue for UVN-T4 group. A significant difference between UVN-NaCl and UVN-T4 group is indicated with * in black (*p < 0.05, **p < 0.01, ***p < 0.001; One-way ANOVA and Tuckey's post tests).

Supplementary Figure 1: L-T4 treatment prevents the positive regulation of SOX2+ cells and increased the number of TR α + cells three days after UVN in the deafferented medial vestibular nucleus. (A) Confocal immunostaining images of TR α + (green) and SOX2+ (red) cells in the deafferented medial vestibular nucleus (MVN) before the lesion (pre-op), three days (D3) or thirty days (D30) after UVN for UVN-NaCl and UVN-T4 groups. (B) Quantitative assessment of the effect of UVN and L-T4 treatment on the number of TR α and Olig2+ cells in the deafferented MVN. Error bars represent SEM. A significant difference from the pre-operative value is indicated by * in red for UVN-NaCl group. A significant difference from the pre-operative value is indicated by * in blue for UVN-T4 group. A significant difference between UVN-NaCl and UVN-T4 group is indicated with * in black (*p < 0.05, **p < 0.01, ***p < 0.001; One-way ANOVA and Tuckey's post tests). Scale bar = 25 μ m.