Bisphenols are important plasticizers currently in use and are released at rates of hundreds of tons each year into the biosphere\textsuperscript{1-3}. However, for any bisphenol it is completely unknown if and how it affects the intact adult brain\textsuperscript{4-6}, whose powerful homeostatic mechanisms could potentially compensate any effects bisphenols might have on isolated neurons. Here we analyzed the effects of one month of exposition to BPA or BPS on an identified neuron in the vertebrate brain, using intracellular in vivo recordings in the uniquely suited Mauthner neuron in goldfish. Our findings demonstrate an alarming and uncompensated in vivo impact of both BPA and BPS—at environmentally relevant concentrations—on essential communication functions of neurons in mature vertebrate brains and call for the rapid development of alternative plasticizers. The speed and resolution of the assay we present here could thereby be instrumental to accelerate the early testing phase of next-generation plasticizers.
Plasticizers are essential ingredients to plastic production\(^7,8\). However, upon degradation of plastic products these additives are released into the environment in large quantity, making plasticizer contamination a serious environmental issue and potential risk for our health\(^1,9–12\). For example, 8 million tons of the plasticizer bisphenol A (BPA; 2,2-bis-(4-hydroxyphenyl)-propane; CAS Registry No. 80-05-7) are produced worldwide each year and 100 tons per year are released into the biosphere\(^2,3\), making BPA ubiquitously present in the environment from surface water to breast milk\(^2\). Initially considered harmless, its various effects on hormonal balance, reproduction, and development in vertebrates\(^5,10,12,15,16\) have led to its replacement—particularly in baby products—by other bisphenols, most notably bisphenol S (BPS; 4,4'-sulfonyldiphenol; CAS Registry No. 80-09-1)\(^4,5\), which is presently available in the EU at rates of 10,000 tons per year\(^17\). Evidence, however, is mounting that also BPS might not be unproblematic\(^1,4,18–24\). Its almost 100-fold higher solubility in water compared to BPA makes BPS now readily detectable in aqueous environments\(^25,26\). Studies in fish models, however, indicate that exposition not only to BPA, but also to BPS results in developmental deformities, impaired and abnormal behavior\(^27–30\).

Here we demonstrate an alarming effect of bisphenols that has, to our knowledge, never been described before: we describe here clear and alarming effects of both BPA and its substitute BPS on neuronal functionality in the mature vertebrate brain despite the powerful homeostatic mechanisms that act in vivo\(^31,32\) and that could in principle compensate for any effects seen in vitro\(^33,34\). Our findings make it very likely that bisphenols also affect the adult human brain and can, among other aspects, change the delicate balance between excitation and inhibition, which is seen as the basis of several neuronal disorders\(^6,35,36\). Our findings call for new approaches to speed up the development and efficient pre-testing of alternative plasticizers. Specifically, the assay that we describe here can rapidly and accurately provide comprehensive information on effects on the mature brain and should therefore be part of a battery of efficient tests in the development of future plasticizers.

Results

Assaying neural function in vivo. To study the effect of exposure to bisphenols on the adult vertebrate brain (Fig. 1a), the Mauthner neuron of fish and some amphibians is an ideal substrate. It is one of the very few neurons in the vertebrate CNS that can be identified individually from one animal to the next and that is readily accessible to intracellular in vivo recording\(^37\). Therefore, it has been a major source of insight into fundamental mechanisms of synaptic communication in the vertebrate CNS\(^38\). The two Mauthner neurons are essential for triggering the vital escape in response to suddenly approaching predators\(^39,40\). This requires the Mauthner neuron to integrate information from all sensory systems. Hence, intracellular recordings from the Mauthner neuron can rapidly and highly sensitively assay a number of key aspects of neuronal and circuit function. In our tests, we elicited action potentials in the Mauthner neurons antidromically (Fig. 1b), i.e., by stimulating their large axons (see “Methods”), and quantitatively analyzed several of its characteristics. Additionally, we presented sensory stimuli to activate visual (Fig. 1c) and acoustic (Fig. 1d) processing and recorded postsynaptic potentials (PSPs) to analyze the integration of sensory information in the Mauthner neuron.

Effects on the action potential. We discovered that even at environmentally relevant concentration of 10 µg L\(^{-1}\) one month of exposure to BPA or BPS massively reduced the maximal initial slope of the action potential (Fig. 2a) in vivo. In the controls (exposed to the solvent DMSO) the maximal initial slope was 2.13 ± 0.33 V ms\(^{-1}\) \((N = 13\) independent animal samples; \(n = 9\) to 31 measurements per fish), 10 µg L\(^{-1}\) BPA reduced it to 0.68 ± 0.44 V ms\(^{-1}\) \((N = 12\) independent animal samples; 76 ≤ \(n\) ≤ 114; one-way ANOVA: \(F = 17.55\); \(R^2 = 0.5899\); \(P < 0.0001\); Dunnett test: mean diff.: 1.39; confidence interval of diff.: 0.95–1.82; \(P < \)
0.0001) and 1 mg L\(^{-1}\) to 1.19 ± 0.22 V ms\(^{-1}\) (\(N = 12\) independent animal samples; 17 ≤ \(n\) ≤ 19; Dunnett test: mean diff.: 0.89; confidence interval of diff.: 0.46–1.33; \(P < 0.0001\)). Exposure to 10 \(\mu\)g L\(^{-1}\) BPS reduced the maximal initial slope of the action potential to 1.20 ± 0.47 V ms\(^{-1}\) (\(N = 11\) independent animal samples; 86 ≤ \(n\) ≤ 104; Dunnett test: mean diff.: 0.80; confidence interval of diff.: 0.35–1.24; \(P < 0.0001\)) and 1 mg L\(^{-1}\) to 1.93 ± 0.62 V ms\(^{-1}\) (\(N = 11\) independent animal samples; 20 ≤ \(n\) ≤ 159; Dunnett test: mean diff.: 0.48; confidence interval of diff.: 0.03–0.92; \(P = 0.032\)). Because we also noticed effects on the time course of the action potential, we analyzed the time-integrated action potential, taking the area \(I_1\) for the first ms after onset (see Fig. 2a). Interestingly, here only the higher plasticizer concentration showed an effect: In controls, \(I_1\) was 23.8 ± 3.1 mV ms (\(N = 13\) independent animal samples; 9 ≤ \(n\) ≤ 31). 1 mg L\(^{-1}\) BPA reduced the integrated action potential in the first ms of its duration to 20.5 ± 3.1 mV ms (\(N = 12\) independent animal samples; 17 ≤ \(n\) ≤ 19; one-way ANOVA: \(F = 6.249\); \(R^2 = 0.3387\); \(P < 0.0001\); Dunnett test: mean diff.: 3.45; confidence interval of diff.: 0.82–6.08; \(P = 0.0055\)). With 1 mg L\(^{-1}\) BPS, \(I_1\) was 19.4 ± 2.9 mV ms (\(N = 11\) independent animal samples; 20 ≤ \(n\) ≤ 159; Dunnett test: mean diff.: 4.09; confidence interval of diff.: 1.40–6.78; \(P = 0.001\)).

**BPA increases neuronal backfiring.** The action potential of the Mauthner neuron can backfire to presynaptic sites through electrical synapses (Fig. 1b). These are part of the mixed “club-ending” synapses that convey acoustic input onto the lateral dendrite of the Mauthner neuron. The resulting depolarization of the presynaptic site can then again cause transmitter release, giving rise to a delayed potential (DP) that lags the action potential by about 1 ms\(^4\). If this also backfires, even a second DP can be generated. The DPs are therefore a valuable tool for assessing how bisphenols affect electrical synapses and presynaptic transmitter release. Figure 2b illustrates two exemplary DPs. They typically followed 0.86 ± 0.06 ms (amplitude 10.3 ± 3.8 mV; \(N = 5\) independent animal samples; \(n = 9\) to 31 measurements per fish; first DP) and 1.44 ± 0.05 ms (amplitude 4.9 ± 1.9 mV; \(N = 4\) independent animal samples; \(n = 9–31\); second DP) after onset of the action potential (Supplementary Fig. 1b). In the control group a pair of DPs occurred consistently in 31% (4 of 13) of the fish, a single DP in 38% (5 of 13). Consistent with the notion that the 2nd DP is caused by transmitter release due to the presynaptic spreading of the 1st DP, we found that the amplitude of the 2nd DP correlates with the amplitude of the first (Supplementary Fig. 1c; \(N = 20\) independent animal samples; 9 ≤ \(n\) ≤ 114; Spearman correlation: \(P = 0.01\)) and was absent when the first one was absent. In contrast, we found no correlation between the amplitude of the 1st DP and the amplitude of the action potential (Supplementary Fig. 1c; \(N = 33\) independent animal samples; 9 ≤ \(n\) ≤ 114; Spearman correlation: \(P = 0.45\)). Strikingly, one month of exposition to BPA strongly affected backfiring in vivo through mixed electrical and chemical synapses. While
both BPA and BPS did not affect the amplitudes of the DPs (Supplementary Fig.1d; one-way ANOVA: $F \leq 1.539; R^2 \leq 0.1751$; $P \leq 0.217$), specifically BPA (but not BPS) dramatically increased the occurrence of DPs: In the group of fish exposed to 1 mg L$^{-1}$ BPA as well as in that exposed to 10 µg L$^{-1}$ BPA the first delayed potential occurred in 75% (9 of 12) (Wilcoxon test for difference from control: $P = 0.009$). An additional second DP occurred in 58% (7 of 12) of fish exposed to 1 mg L$^{-1}$ BPA (Wilcoxon test for difference from control: $P = 0.086$) and in 33% (4 of 12) of fish exposed to 10 µg L$^{-1}$ BPA (Wilcoxon test for difference from control: $P = 0.13$). BPA thus strongly increased neuronal back-firing. In light of the findings below, this is a likely consequence of increased transmission at the glutamatergic mixed synapses and increased spreading of the action potential to presynaptic sites.

Bisphenols affect acoustic processing. One month of exposure to BPA or BPS had striking and uncompensated effects on the PSPs that were elicited by our broadband acoustic pulse. The experimental setting and an exemplary PSP of a control animal are shown in Fig. 3a, b. Strikingly, the bisphenols affected basically all aspects of the acoustic PSP. BPA and BPS both increased the amplitude, the temporal integral and its longtime decay. Maximum amplitude of the PSPs was increased from 7.1 ± 1.4 mV ($N = 13$ independent animal samples; between $n = 8−29$ measurements per fish) in the controls to 11.2 ± 3.0 mV ($N = 11$ independent animal samples; $16 \leq n \leq 49$) with 10 µg L$^{-1}$ BPA and to 10.4 ± 2.3 mV ($N = 11$ independent animal samples; $9 \leq n \leq 46$) with 1 mg L$^{-1}$, 10 µg L$^{-1}$ BPA increased the maximum amplitude to 10.2 ± 2.1 mV ($N = 12$ independent animal samples; $10 \leq n \leq 52$) and 1 mg L$^{-1}$ to 11.4 ± 1.9 mV ($N = 12$ independent animal samples; $11 \leq n \leq 23$). With respect to the first delayed potential, we considered the temporal integral in four consecutive intervals, 50 ms each (Fig. 3b; integrals $I_1$ to $I_4$). This analysis showed a clear increase of the first integral (Fig. 3c; one-way ANOVA: $F = 6.479; R^2 = 0.3396; P < 0.0001$) from 117.5 ± 32.1 mV ms ($N = 13$ independent animal samples; $8 \leq n \leq 29$) in the controls to 173.6 ± 32.6 mV ms ($N = 11$ independent animal samples; $16 \leq n \leq 49$) with 10 µg L$^{-1}$ BPS (Dunnnett test: mean diff.: $−53.74$; confidence interval of diff.: $−91.16$ to $−16.32; P = 0.002$) and to 188.7 ± 47.2 mV ms ($N = 11$ independent animal samples; $9 \leq n \leq 46$) with 1 mg L$^{-1}$ BPS (Dunnnett test: mean diff.: $−67.47$; confidence interval of diff.: $−104.9$ to $−30.05; P < 0.0001$). BPA significantly increased $I_1$ in high (Dunnnett test: mean diff.: $−63.64$; confidence interval of diff.: $−100.20$ to $−27.07; P = 0.0001$), but not in low concentration (Dunnnett test: mean diff.: $−25.96$; confidence interval of diff.: $−62.53$ to $10.61; P = 0.2516$).

In conclusion, both bisphenols had striking effects on almost all functionally relevant aspects of the acoustic PSP. Most remarkably they increased the efficiency at which the acoustic stimulus excited the Mauthner neuron. Because at least BPS is thought to negatively affect sensory hair cells$^{42}$, a decrease rather than an increase of the amplitude of acoustic PSPs would have been expected. Our findings, therefore, suggest important and unbalanced excitatory effects of BPA and BPS on (glutamatergic$^{41}$) synaptic transmission in the CNS.

Bisphenols affect visual processing. The bisphenols not only affected acoustic circuits but had striking effects on the visual PSP. The experimental setting and an exemplary PSP are shown in Fig. 4a, b. In contrast to their effect on the acoustic PSP, BPA and BPS strongly reduced the amplitude of the visual PSPs (Fig. 4c; one-way ANOVA: $F = 17.83; R^2 = 0.6058; P < 0.0001$; Dunnnett test: $P \leq 0.0046$). In controls, PSP amplitude was 10.4 ± 1.8 mV ($N = 8$ independent animal samples; $n = 7$ to 21 measurements per fish). 10 µg L$^{-1}$ BPA reduced it to 2.9 ± 1.5 mV ($N = 12$ independent animal samples; $8 \leq n \leq 27$) and 1 mg L$^{-1}$ BPA to 6.3 ± 1.5 mV ($N = 12$ independent animal samples; $10 \leq n \leq 21$). In BPS exposed fish, PSP amplitude was 2.5 ± 2.4 mV ($N = 11$ independent animal samples; $7 \leq n \leq 31$) for 10 µg L$^{-1}$ and 5.0 ± 3.4 mV ($N = 11$ independent animal samples; $11 \leq n \leq 25$) for 1 mg L$^{-1}$ BPS. In addition, 1 mg L$^{-1}$ BPA (but not the low concentration of BPA tested or BPS) also drastically reduced the maximal initial slope of the PSPs from 3.66 ± 0.82 mV ms$^{-1}$ ($N = 8$ independent animal samples; $7 \leq n \leq 21$) to only 0.32 ± 0.07 mV ms$^{-1}$ ($N = 12$ independent animal samples; $10 \leq n \leq 21$; one-way ANOVA: $F = 24.51; R^2 = 0.6788; P < 0.0001$; Dunnnett test: mean diff.: $3.00$; confidence interval of diff.: $2.04$ to $3.96; P < 0.0001$). BPA and BPS additionally affected the temporal integral of the PSPs (Fig. 4c; one-way ANOVA: $F = 11.43; R^2 = 0.4964; P < 0.0001$) with the first integral (of 75 ms duration) strongly
decreased from 398.0 ± 71.5 mV ms (N = 8 independent animal samples; 7 ≤ n ≤ 21) in the control group to 169.5 ± 107.8 mV ms with 10 µg L⁻¹ BPA (N = 12 independent animal samples; 8 ≤ n ≤ 27; Dunnett test: mean diff.: 205.4; confidence interval of diff.: 83.8–327.1; P = 0.0003), to 150.9 ± 82.8 mV ms with 10 µg L⁻¹ BPS (N = 11 independent samples; 7 ≤ n ≤ 31; Dunnett test: mean diff.: 227.1; confidence interval of diff.: 103.3–350.9; P < 0.0001) and to 250.0 ± 125.4 mV ms with 1 mg L⁻¹ BPS (N = 11 independent animal samples; 11 ≤ n ≤ 25; Dunnett test: mean diff.: 134.3; confidence interval of diff.: 10.5–258.2; P = 0.0293).

**Effects of EE2.** For many of the varied non-neuronal effects of bisphenols their structural similarity with estrogens is crucial. A series of experiments was therefore aimed at exploring whether this might also apply, to some extent, to the strong neuronal effects we describe here. We therefore ran experiments just as with BPA and BPS (Figs. 2–4) and also with one month of exposure, but with fish exposed not to any bisphenols but to ethinyl estradiol (EE2) at a concentration of 1 mg L⁻¹. A full account of all results obtained in these experiments is given in Supplementary Table 1. Table 1 highlights all...
Significant effects that we were able to detect with EE2 and compares their occurrence and direction with those we found after BPA and BPS exposition (at any concentration). EE2 highly significantly increased action potential amplitude and shortened the delay, after which an action potential followed after spinal cord stimulation, effects that we found neither with BPA nor BPS at any concentration. However, all other effects, including their direction, were strikingly similar as with the bisphenols. This might suggest that at least some of the neuronal effects of the bisphenols could also result from their similarity with estrogens.

Acute effects of BPA and BPS. One month of exposition to BPA or BPS at concentrations of 10 µg L\(^{-1}\) or 1 mg L\(^{-1}\) caused strong effects on all aspects of neuronal function. Our final series of experiments was therefore aimed at testing whether the effects required prolonged exposition or might at least partly be seen in acute experiments. In these, the tests shown in Figs. 2–4 were run for a total of 20 min in untreated fish, to establish baseline properties. Then either BPS (\(N = 6\) independent animal samples) or BPA (\(N = 7\) independent animal samples) was added so that the fish now faced a concentration of 10 µg L\(^{-1}\). After 10 min of incubation the 20 min stimulus program was run again. Subsequently, the concentration of the respective bisphenol was increased to 1 mg L\(^{-1}\) and an incubation of 10 min was allowed before the stimulus program was given. At the measurements at the higher concentration, the fish had been exposed to bisphenol for a comparably brief time between 40 min (10 min at the high concentration plus 30 min at the lower concentration) and 60 min. The results of all three series (baseline, 10 µg L\(^{-1}\) BPA, 1 mg L\(^{-1}\) BPA; 10 µg L\(^{-1}\) BPS, 1 mg L\(^{-1}\) BPS) are reported in detail in Supplementary Table 2. In none of the experiments did the acute exposition cause any significant deviations from baseline (RM one-way ANOVA: \(F \leq 3.59; R^2 \leq 0.42; P \geq 0.07\)). These findings therefore suggest that the strong neuronal effects seen after one month of exposure do not establish quickly after short exposure of only about 1 h.
Discussion

Our in vivo recordings demonstrate strikingly strong and uncompensated effects of bisphenols on all aspects of neuronal function in the adult vertebrate brain, from the action potential, the balance between excitatory and inhibitory inputs to auditory and visual sensory circuits. Our findings have been obtained in a particularly accessible identified neuron in the mature vertebrate CNS, the Mauthner neuron of the goldfish. This neuron is particularly interesting for an analysis of whether the effects of bisphenols could be buffered: Buffering should be particularly strong in this neuron, because its inputs and outputs are essential for driving life-saving escapes. Although the effects of bisphenols certainly vary between individual neurons and across species, our findings clearly establish that the effects of bisphenols on the nervous system are by no means restricted to developing brains. Rather, being exposed to either BPA or BPS at the environmentally relevant concentration of 10 µg L−1 for one month strongly affects neuronal function in the adult brain.

On the more optimistic side, our findings demonstrate that it is possible to quickly gain sensitive information on basic neuronal functions—from generation of the action potential, synaptic transmission to auditory and visual function—by using multisensory integration in identified neurons such as the Mauthner neuron as a powerful tool. Studying the postsynaptic potentials in response to acoustic or visual stimulation showed clear effects of both bisphenols on sensory systems and on central processing. Although it has been suggested that BPA damages sensory hair cells in fish and amphibia, we find that BPA—surprisingly—increased the amplitude of acoustical PSPs and that BPS acted similarly. These effects could be explained by a strong effect of both BPA and BPS on excitatory synaptic transmission. However, our findings also demonstrate that not all synapses are equally potentiated: For instance, backfiring through the mixed synapses was strongly increased by BPA, but not affected by BPS. Furthermore, the visual PSPs were clearly reduced both after exposure to BPA or BPS, which would only for BPS be attributable to an effect on retinal function.

The strong effects we find here and the apparent lack of efficient buffering are alarming. The effects of bisphenols have previously been discussed mainly from a developmental point of view (causing the ban of BPA from baby products in some countries) or from its varied endocrinological effects. Now we face an additional danger whose effect on healthy humans and on patients with neurological deficits is difficult to foresee. Offsetting balances in brains is the basis of severe neurological disorders and so our findings must be taken very seriously. What is most needed, is an effort to develop a new generation of plasticizers combined with an efficient but sufficiently broad and sensitive array of tests to quickly detect and sort out substances that bear large environmental and health risks.

The tests we described here are particularly efficient and can quickly assay effects on neuronal functions. Together with similarly sensitive assays they could guide our way to the urgently needed next-generation plasticizers.

Methods

Animals and treatment. We used N = 98 goldfish (Carassius auratus, Cypriniformes) of either sex with an average standard length of 69.5 ± 7.8 mm (range from 56.3 to 100 mm) and an average body weight of 10.3 ± 3.8 g (range from 6.7 to 20.8 g). The fish were obtained from an authorized specialist retailer (Aquarium Glaser GmbH, Rodgau, Germany). Prior to the start of the project, fish were kept for at least 4 weeks in large glass tanks (250 × 50 × 50 cm) filled with fresh water (water conductivity: 300 ± 7 µS cm−1; pH 7.5; total hardness of water: 7.7°DH; NH4− < 10 µg L−1; NO3− < 5 µg L−1; NO2− < 5 µg L−1) at a water temperature of 20 °C. Light/dark photoperiod was 12:12 h. Fish were fed once a day with common fish food (sera gold; sera GmbH, Heinsberg, Germany). After this period of acclimatization and quarantine, fish were checked for disorders and for responsiveness to visual and acoustic stimuli. We only chose healthy and responsive fish for the experiments. They were divided randomly into experimental groups exposed either to bisphenol A (BPA; 4,4′-(propane-2,2-diyl)-diphenol), bisphenol S (BPS; 4,4′-sulfonyldiphenol) or to ethinyl estradiol (EE2; 17α-ethinyl-1,3,5(10)-oestren-3,17β-diol). BPA and BPS were obtained in granular form from Sigma-Aldrich (Steinheim, Germany), while EE2 was obtained in powder form from Merck KGaA (Darmstadt, Germany). For application, they were dissolved in dimethyl sulfoxide (DMSO), with a final DMSO concentration of 0.01% and added in the required concentration to the water.

Two experimental groups (7 fish each) were used to test for acute effects of BPA and BPS. Fish of these groups were not exposed to plasticizer prior to experiment. However, during Mauthner neuron intracellular recording, we added plasticizer (BPA or BPS) so that the fish acutely faced either BPA or BPS. Thereby, we were able to collect robust data for two concentrations (10 µg L−1 and 1 mg L−1) in N = 7 fish of the BPA group and N = 6 fish of the BPS group.

In six further groups (14 fish each), we tested for effects of BPA, BPS, and EE2 after a month of exposition. Fish of these groups were, respectively, exposed either to 10 µg L−1 BPA, 1 mg L−1 BPA, 10 µg L−1 BPS, 1 mg L−1 BPS, 1 mg L−1 EE2 or received only DMSO in the concentration used as solvent in the other groups. The latter group served as a control. By starting exposition at different times, each experimental fish was exactly dosed to the respective chemical for 30 to 33 days. We were able to collect robust data in N = 13 fish of the control group, N = 12 fish of the 10 µg L−1 BPA group, N = 12 fish of the 1 mg L−1 BPA group, N = 11 fish of the 10 µg L−1 BPS group, N = 11 fish of the 1 mg L−1 BPS group and N = 10 fish of the 1 mg L−1 EE2 group. Two fish exposed to EE2 died prior experiment in the third week of exposition. Animal care procedures, surgical procedures, and experimental procedures were in accordance with all relevant guidelines and regulations of the German animal protection law and explicitly approved by state councils (Regierung von Unterfranken, Würzburg, Germany).

Anesthesia and surgical procedure. Before starting surgery, the experimental fish was anaesthetized (2-phenoxethanol in the concentration of 0.4 mL l−1) for 15 min in the water it was used to. Anaesthesia was maintained also during surgery and during recording and the protocol is known not to affect neuronal functionality nor the acoustical or the visual system of goldfish. To confirm the sufficiency of anesthesia, we carefully exerted pressure to the fish’s caudal peduncle after the fish had lost equilibrium, which normally would trigger vigorous escapes. Only when this stimulation (and subsequent handling) yielded no response, the fish was positioned in the recording chamber and given artificial respiration with aerated, anesthetic loaded water flowing via a tube through the fish’s mouth and out over the gills at a flow rate of 80 ml min−1. Here, we also used water of the same quality as for housing. Respiratory water was delivered to the fish from a reservoir using a suitably adjusted pump (EHEIM universal 300; EHEIM GmbH & Co. KG, Deizisau, Germany) at a regular power of 4.8 l h−1.

Access to the Mauthner neurons was achieved by using a bone rongeur to open the skull from above in the area of the hindbrain. To expose the medulla oblongata

### Table 1 The spectrum of significant effects on neuronal function found after one month of exposition to either ethinyl estradiol (EE2) at 1 mg L−1 or bisphenols (BPA or BPS) at any of the concentrations we examined (10 µg L−1 and 1 mg L−1).

| Substance | EE2 | BPA | BPS |
|-----------|-----|-----|-----|
| Effect on antidromically induced action potential | | | |
| Amplitude | ↓ | ↓ | ↓ |
| Delay | ↓ | ↓ | ↓ |
| Slope | ↓ | ↓ | ↓ |
| Area | ↓ | ↓ | ↓ |
| 1st DPs | ↑ | ↑ | ↑ |
| Effect on auditory induced PSPs | | | |
| Amplitude | ↑ | ↑ | ↑ |
| Delay | ↓ | ↓ | ↓ |
| Slope | ↓ | ↓ | ↓ |
| Area | ↓ | ↓ | ↓ |
| Effect on visually induced PSPs | | | |
| Amplitude | ↓ | ↓ | ↓ |
| Delay | ↓ | ↓ | ↓ |
| Slope | ↓ | ↓ | ↓ |
| Area | ↓ | ↓ | ↓ |

Based on data shown in Figs. 2–4 and Supplementary Table 1. 

Indicate a significant decrease in comparison to control and 1 a significant increase; free fields represent values that have not changed significantly in comparison to control.
containing the pair of Mauthner neurons, the cerebellum was lifted up with a piece of filter paper and fixed in place. To stimulate the axons of the two Mauthner neurons, we additionally exposed a piece of the spinal column (about 5 mm in length) from the side in the region of the trunk (between 20 and 25 mm caudal from the position of the Mauthner somata) and confirmed suprathreshold stimulation of the Mauthner axons from the characteristic twitching of the experimental animal. To prepare for the intracellular in vivo recording the experimental animal was then immobilized by injecting d-tubocurarine (1 µg g⁻¹ body weight; Sigma-Aldrich, Steinheim, Germany) in the core muscles. After finishing measurements, the experimental animal was sacrificed immediately and without recovery from anesthesia by mechanically destroying the brain. Finally, a necropsy was performed to check for any unnoticed diseases of inner organs. This confirmed that all fish of this study were healthy.

**Experimental procedure.** For intracellular recordings, we used a bridge-mode amplifier (BA-01X; npi electronic GmbH, Tann, Germany) in current clamp mode. Recording electrodes were pulled from 3 mm-glass capillaries (G-3; Narishige Scientific Instrument Lab, Tokyo, Japan) using a vertical electrode puller (PE-22: Narishige International Limited, London, UK). Filled with 5 M potassium acetate, they had a resistance between 4 and 7 MΩ. For moving and positioning the recording electrode, we used a motorized micromanipulator (MP-285; Sutter Instrument, Novato, CA, USA). We used established techniques to determine recording position from extracellular space and to ensure recordings are always taken in the soma of the Mauthner neuron. The reference electrode was positioned directly in front of the ipsilateral eye. The light stimulus was a neutral density filter paper and fixed in place. To stimulate the axons of the two Mauthner neurons, we used a short acoustic broadband pulse (duration 1 ms; frequency distribution from 25 to 1000 Hz; peak amplitude at 300 Hz) with a sound pressure level (SPL) of 145 dB re 1 µPa. We measured SPL under water at the position of the fish in the recording chamber with a hydrophone (Type 8106; Bruel & Kjær, Naerum, Denmark). For visual stimulation, we used a light-emitting diode (LED; RS Components GmbH, Mönchengladbach, Germany), which was positioned directly in front of the ipsilateral eye. The light flash used for visual stimulation had a duration of 7 ms. LED peak radiation at 569 nm was 700 µW m⁻² nm⁻¹ and the width at 100 µW m⁻² nm⁻¹ was 56 nm (range: 543–599 nm).

In experiments on the acute effect of plasticizers, each fish was given the set of stimuli three times. The first set of stimuli was presented 10 min after establishing intracellular Mauthner neuron recording and before adding any plasticizer and served to establish a baseline. Next, we added plasticizer (either BPA or BPS) to the water to reach a concentration of 10 µg L⁻¹. After an incubation period of 10 min we recorded Mauthner neuron responses to our set of stimuli again. Then, we increased the concentration to 1 mg L⁻¹ and repeated the protocol (about 10 min before taking the final measurement). In total, all measurements were completed within 90 min of intracellular recording, and the maximum time any bisphenol could have acted in our acute experiments was 60 min. In fish exposed for a month to either BPA, BPS or EE2 we presented our set of stimuli 10 min after establishing intracellular Mauthner neuron recording only once. Per fish we needed 30 min of intracellular recording.

**Statistics and reproducibility.** Statistical tests were run using the software package GraphPad Prism 8.2.1 (GraphPad Software, Inc., La Jolla, CA, USA) and performed two-tailed with α = 0.05. Averages are reported as median ± standard deviation. N denotes the number of independent animal samples, n the number of measurements per animal. When data from animals were pooled, we never used the mean deviation repetitions (n) taken from the individual animals but a single averaged value for each animal. To determine whether there are acute effects of BPA and BPS, we used RM one-way ANOVAs. To determine whether there is an effect of 1-month exposure to BPA or BPS in comparison to the control group, we performed one-way ANOVAs and the Dunnett test for comparing each group with control. To determine whether there is an effect of one month exposure to EE2 in comparison to the control group, we performed unpaired t tests. Rate constants of exponential decay were compared using one-way ANOVA and Dunnett test. Differences in occurrence (in %) were compared using the Wilcoxon test with occurrence for control set as the hypothetical value. To test whether there are correlations between action potential amplitude and DP amplitude and between DP amplitudes, we used Spearman tests. To test whether data is distributed normally, we used the Shapiro–Wilk test.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

The datasets generated and/or analyzed are available from the corresponding author on reasonable request. The source data for the graphs and charts in the main figure are present in the Supplementary data files.

Received: 15 August 2020; Accepted: 8 March 2021;
Published online: 12 April 2021

**References**

1. Chen, D. et al. Bisphenol analogues other than BPA: environmental occurrence, human exposure, and toxicity—a review. Environ. Sci. Technol. 50, 5438–5453 (2016).
2. Wu, L.-H. et al. Occurrence of bisphenol S in the environment and implications for human exposure: a short review. Sci. Total Environ. 615, 87–98 (2018).
3. Li, J. et al. Transformation of bisphenol AF and bisphenol S permanganate in the absence/presence of iodide: kinetics and products. Chemosphere 217, 402–410 (2019).
4. Glausiuz, J. The plastics puzzle. Nature 508, 306–308 (2014).
5. Zimmerman, J. B. & Anastas, P. T. Toward substitution with no regrets. Science 347, 1198–1199 (2015).
6. Tshala-Katumbay, D., Mwanza, J.-C., Rohlmans, D. M., Maestre, G. & Oria, R. A. Global perspective on the influence of environmental exposures on the nervous system. Nature 527, S187–S192 (2015).
7. Hermabessiere, L. et al. Occurrence and effects of plastic additives on marine environments and organisms: a review. Chemosphere 182, 781–793 (2017).
8. Halaladakis, J. N., Velis, C. A., Weber, R., Iacovidou, E. & Purnell, P. An overview of chemical additives present in plastics: Migration, release, fate and environmental impact during their use, disposal and recycling. J. Hazard. Mater. 344, 179–199 (2018).
9. Kaiser, J. Controversy continues after panel rules on bisphenol A. Science 317, 884–885 (2007).
10. Borrell, B. The big test for bisphenol A. Nature 464, 1122–1124 (2010).
11. Fagin, D. The learning curve. Nature 490, 462–465 (2012).
12. Flint, S., Markle, T., Thompson, S. & Wallace, E. Bisphenol A exposure, effects, and policy. Environ. Manag. 104, 19–34 (2012).
13. Vandenberg, L. N. et al. Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A. Environ. Health Persp. 118, 1055–1070 (2010).
14. Jiang, D., Chen, W.-Q., Zeng, X. & Tang, L. Dynamic stocks and flows analysis of bisphenol A (BPA) in China: 2000–2014. Environ. Sci. Technol. 52, 7076–7115 (2018).
15. Howdeshell, K. L., Hotchkiss, A. K., Thyayer, K. A., Vandenbergh, J. G. & vom Saal, F. S. Exposure to bisphenol A advances puberty. Nature 401, 763–764 (1999).
16. Heindel, J. J., Newbold, R. & Schug, T. T. Endocrine disruptors and obesity. Nat. Rev. Endocrinol. 11, 306–313 (2015).
17. Choi, Y. J. & Lee, L. S. Aerobic soil biodegradation of bisphenol (BPA) alternatives bisphenol S and bisphenol BPA compared to BPA. Environ. Sci. Technol. 51, 13698–13704 (2017).
18. Helies-Toussaint, C., Peyre, L., Costanzo, C., Chagnon, M. C. & Rahmani, R. Exposure to bisphenol A advances puberty. Proc. Natl Acad. Sci. USA 112, 1475–1480 (2015).
19. Rosenmai, A. K. et al. Are structural analogues to bisphenol A safe alternatives? Toxicol. Sci. 139, 35–47 (2014).
20. Kinch, C. D., Ibaheziehbo, K., Jeong, J.-H., Habibi, H. R. & Kurrasch, D. M. Low-dose exposure to bisphenol A and replacement bisphenol S induces precocious hypothalamic neurogenesis in embryonic zebrafish. Proc. Natl Acad. Sci. USA 112, 5482–5487 (2015).
21. Yamazaki, E. et al. Bisphenol A and other bisphenol analogues including BPS and BPF in surface water samples from Japan, China, Korea and India. Ecotox. Environ. Safet. 122, 565–572 (2015).
22. Kolla, S., McSweeney, D. B., Pohkarel, A. & Vandenbarg, L. N. Bisphenol S alters development of the male mouse mammary gland and sensitizes it to a peripubertal estrogen challenge. Toxicology 424, 152234 (2019).

23. Zhang, X., Li, C., Pan, J., Liu, R. & Cao, Z. Searching for a bisphenol A substitute: effects of bisphenols on catalase molecules and human red blood cells. Sci. Total Environ. 669, 112–119 (2019).

24. Salahinejad, A. et al. Effects of chronic exposure to bisphenol-S on social behaviors in adult zebrafish: Disruption of the neuropeptide signaling pathways in the brain. Environ. Pollut. 262, 113992 (2020).

25. Guo, H. et al. Structural benefits of bisphenol S and its analogs resulting in their high sorption on carbon nanotubes and graphite. Environ. Sci. Pollut. Res. 23, 8976–8984 (2016).

26. Fang, Z. et al. A critical review on remediation of bisphenol S (BPS) contaminated water: efficiency and mechanisms. Crit. Rev. Environ. Sci. Tech. 50, 1628902 (2020).

27. Saili, K. S. et al. Neurodevelopmental low-dose bisphenol A exposure leads to early-life stage hyperactivity and learning deficits in adult zebrafish. Toxicology 291, 83–92 (2012).

28. Gu, J. et al. Neurobehavioral effects of bisphenol S exposure in early life stages of zebrafish larvae (Danio rerio). Chemosphere 217, 629–635 (2019).

29. Kim, S. S. et al. Neurochemical and behavioral analysis by acute exposure to bisphenol A in zebrafish larvae model. Chemosphere 239, 124751 (2019).

30. Naderi, M., Salahinejad, A., Attaran, A., Chivers, D. P. & Niyogi, S. Chronic exposure to environmentally relevant concentrations of bisphenol S differently affects cognitive behaviors in adult female zebrafish. Environ. Pollut. 261, 114060 (2020).

31. Davis, G. W. Homeostatic signaling and the stabilization of neural function. Neuron 80, 718–728 (2013).

32. Keck, T. et al. Synaptic scaling and homeostatic plasticity in the mouse visual cortex in vivo. Neuron 80, 327–334 (2013).

33. Zhou, R. et al. Abnormal synaptic plasticity in basolateral amygdala may account for hyperactivity and attention-deficit in male rat exposed perinatally to low-dose bisphenol-A. Neuropharm 60, 789–798 (2011).

34. Hu, F. et al. Bisphenol A impairs synaptic plasticity by both pre- and postsynaptic mechanisms. Adv. Sci. 4, 1600493 (2017).

35. Ramocki, M. B. & Zoghbi, H. Y. Failure of neuronal homeostasis results in common neuropsychiatric phenotypes. Nature 455, 912–918 (2008).

36. Nelson, S. B. & Valakh, V. Excitatory/inhibitory balance and circuit homeostasis in autism spectrum disorders. Neuron 87, 684–698 (2015).

37. Furshpan, E. J. & Furukawa, T. Intracellular and extracellular responses of the integrating central neuron provide a quick way for identifying appropriate anaesthetic use in fish. Sci. Rep. 8, 175541 (2018).

38. Machnik, P., Schirmer, E., Glück, L. & Schuster, S. Recordings in an anaesthetic use in integrating central neuron provide a quick way for identifying appropriate anaesthetic use in fish. Sci. Rep. 8, 17541 (2018).

39. Hecker, A., Schulze, W., Oster, J., Richter, D. O. & Schuster, S. Removing a material in this article are included in the article Commons license, and indicate if changes were made. The images or other third party material is this article are included in the article Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.