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# Modulatory effect of diphenyl diselenide in Carioca High- and Low-conditioned Freezing rats

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ABSTRACT

Diphenyl diselenide ($[PhSe]_2$)is an organoselenium compound that has interesting pharmacological properties, including antioxidant, glutathione peroxidase-mimetic, and neuroprotective effects. The objective of the present study was to investigate the possible modulatory effect of $(PhSe)_2$ in 17th-generation Carioca high-and low-conditioned freezing (CHF and CLF) rats, an animal model of generalized anxiety disorders. $(PhSe)_2$ was administered at three doses (10, 50, and 100 mg/kg) in CHF and CLF rats, and their anxiety-like profiles (conditioned freezing patterns) were measured before and 30 min after treatment. A significant difference was found in freezing scores between CHF and CLF animals before treatment ($t_{70}=12.50$, $p<0.001$). Treatment with $(PhSe)_2$ at 10 and 50 mg/kg decreased freezing in CHF rats but significantly increased freezing at 100 mg/kg. $(PhSe)_2$ increased freezing in CLF animals at 50 and 100 mg/kg ($p<0.01$). These results indicate that $(PhSe)_2$ exerts both anxiolytic- and anxiogenic-like effects in bi-directional rat lines. Distinct genetic profiles of the CHF and CLF lines may influence biochemical functions and lead to differential responses to aversive situations and various drugs like $(PhSe)_2$.



## 1. Introduction

We recently demonstrated an association between oxidative stress and the genesis of anxiety using a novel rat breeding line known as Carioca High-and Low-Conditioned Freezing (CHF and CLF; Hassan et al., 2013). The breeding protocol for these animals is based on defensive freezing responses to contextual cues that are associated with electric footshock (Dias et al., 2009; Castro-Gomes and Landeira-Fernandez, 2008). The anxiety-like profile of these animals was confirmed with several behavioral test protocols (Hassan et al., 2013; Dias et al., 2009; Castro-Gomes and Landeira-Fernandez, 2008). The levels of reactive species (RS) and rate of lipid peroxidation (LPO) were higher in CHF rats than in CLF rats. Consequently, low antioxidant enzymatic status was confirmed in CHF animals, reflected by glutathione peroxidase (GPx) and catalase (CAT) activity in various brain structures, including the cortex, hippocampus, and cerebellum (Hassan et al., 2013). These results are consistent with other studies that reported the direct involvement of oxidative stress in anxiety-related disorders (Salim, 2011). Oxidative stress has been linked to the pathological manifestations of other psychological and neurological disorders (Andersen, 2004).

The use of antioxidants may be beneficial for ameliorating the adverse effects of oxidative bursts and may help reduce anxiety. Various dietary and synthetic antioxidants have been reported to protect against anxiety-related disorders (Salim, 2011; Bouayed and Kalinin, 2011; Augustyniak et al., 2010). Vitamin C, rutin, caffeic acid, and rosmarinic acid have been reported to have antidepressant and anxiolytic effects at lower doses (Atmaca et al., 2004). Masood et al. (2008) recently showed that supplementation with tempol (an antoxidant) reduced buthionine-[*S*,*R*]-sulfoximine (BSO)-induced anxiety-like behavior in rats. Importantly, BSO produces oxidative stress by inhibiting glutathione (GSH) synthesis.

Data regarding synthetic antioxidants are very diverse, and a range of different classes have been reported in the literature (Augustyniak et al., 2010). Organoselenium compounds have gained increasing attention because of their broad applications in organic synthesis and pharmacological efficacy. Various classes of selenium compounds have been shown to have interesting

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biological effects, including glutathione peroxidase-mimetic, lipid peroxidation, radical-scavenging, antiinflammatory, antinociceptive, cardioprotective, and thioredoxin reductase activity (Nogueira et al., 2004). Diphenyl diselenide ($[PhSe]_2$), the simplest diaryl diselenide, has shown remarkable potential in various animal models of pathology (Nogueira and Rocha, 2010). Interestingly, selenium deficiency has been related to depression, mood disorders, and anxiety (Sher, 2000; 2007; Rayman, 2000).Selenium supplementation has been shown to improve both anxious and depressive symptoms (Benton, 2002; Benton and Cook, 1991). Different mono- and diselenides have been used in various models of anxiety and depression. Depending on the chemical structure, route of administration, and dose, various compounds have shown promising antidepressant and anxiolytic activities (Nogueira and Rocha, 2011).

To our knowledge, no study has focused on the possible modulatory effects of $(PhSe)_2$in bi-directional rat lines. With distinct and specific neurochemical and neurogenetic profiles, bi-directional lines are important for exploring the underlying mechanisms of anxiety-related behavior. In the present study, 17th-generation CHF and CLF rats were treated with different doses of $(PhSe)_2$ to gain a better understanding of the possible psychomodulatory effects of this organoselenium compound.

## 2. Material and methods

### 2.1. Animals

The present study used rats that were selectively bred for high (CHF) and low (CLF) contextual fear conditioning according to procedures described in our previous work (Castro-Gomes and Landeira-Fernandez, 2008). Female albino Wistar rats from the 17th generation of selective breeding were 15–20 weeks old and weighed 180–300 g at the beginning of the study. They were bred and maintained in the colony room in the Psychology Department of the Pontifícia Universidade Católica do Rio de Janeiro with controlled room temperature ($24 \pm 1$ °C) and a 12 h/12 h light/dark cycle (lights on 7:00 AM–7:00 PM). The animals were housed in groups of three to five, according to their respective lines, in polycarbonate cages ($18 \times 31 \times 38$ $cm^3$) with food and water available *ad libitum*. All of the behavioral experiments were conducted during the light phase of the light/dark cycle. The animals were handled once daily for a period of 2 min for 5 days before the fear conditioning experiment. The experimental procedures reported herein were performed in accordance with the guidelines for experimental animal research established by the Brazilian Society of Neuroscience and Behavior (SBNeC) and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Animal handling and the methods of sacrifice were reviewed and approved by the Committee for Animal Care and Use of PUC-Rio (protocol no. 20/2009).

### 2.2. Chemicals

$(PhSe)_2$ was prepared in our laboratory according to a previous report (Paulmier, 1986). Analysis of the $^1H$ nuclear magnetic resonance (NMR) and $^{13}C$ NMR spectra showed that $(PhSe)_2$ presented analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of the compound (99.9%) was determined by gas chromatography/high-performance liquid chromatography and was stable under storage (room temperature, humidity, and light) conditions.

### 2.3. Preparation of $(PhSe)_2$ solution

The compound was dissolved in canola oil, and the desired concentrations of $(PhSe)_2$ were analyzed and prepared prior to use. The solutions were stored at 2–8 °C and allowed to warm to room temperature before use. The adult rats were given a single i.p. injection of 10, 50, or 100 mg/kg $(PhSe)_2$. The dosages of $(PhSe)_2$that were used in thepresent study were within the therapeutic range (Nogueira and Rocha, 2011) because $(PhSe)_2$ causes neurotoxicity at very high doses.

### 2.4. Apparatus

Contextual fear conditioning occurred in four observation chambers ($25 \times 20 \times 20$ $cm^3$). Each observation chamber was placed inside a sound-attenuating box. A red light bulb (25 W) was placed inside the box, and a video camera was mounted to the back of the observation chamber so the animal's behavior could be observed on a monitor outside the experimental chamber. The floor of each observation chamber was composed of 15 stainless-steel rods (4 mm diameter) spaced 1.5 cm center-to-center, which were wired to a shock generator and scrambler (AVS, SCR04; São Paulo, Brazil). An interface with eight channels (Insight, Ribeirão Preto, Brazil) connected the shock generator to a computer, which allowed the experimenter to apply an electric footshock. A digital multimeter was used to calibrate the shock intensities before each experiment. An ammonium hydroxide solution (5%) was used to clean the chamber before and after each test.

### 2.5. Procedure

During the acquisition phase, each animal was placed in the observation chamber for 8 min. At the end of this period, three 0.6 mA unsignaled electric footshocks were delivered (1 s duration, 20 s intershock interval). The animals were then returned to their home cage 3 min after the last shock. The next day, the animals were returned to the same chamber for 8 min with no footshock or other stimulation during this period for the phenotyping test session. A time-sampling procedure was used to assess fear conditioning in response to contextual cues. Every 2 s, the animal was observed, and a well-trained observer recorded episodes of freezing, which were defined as the total absence of movement of the body or vibrissa, with the exception of movement required for respiration. One week after this initial test session of contextual aversive conditioning (phenotyping), CHF and CLF rats were tested again in the same observation chamber. Thirty minutes before the test, four independent groups of CHF rats and four independent groups of CLF rats were intraperitoneally injected with either $(PhSe)_2$ at three different concentrations (10, 50, and 100 mg/kg) or vehicle. After the injections,all of the rats were returned to their home cages where they remained before the behavioral test session. The same time-sampling procedure described above was used to record freezing behavior during the 8 min test session.

## 3. Results

Fig. 1 shows the mean and standard error of the mean (SEM) percentage of time spent freezing in CHF and CLF animals of the 17th generation during the contextual fear conditioning test session. As expected, CHF animals froze more that CLF animals. This difference was confirmed statistically. Student's *t*-test revealed a significant difference between CHF and CLF animals ($t_{70} = 12.50$, $p < 0.001$).

Fig. 2 shows the mean and SEM percentage of time spent

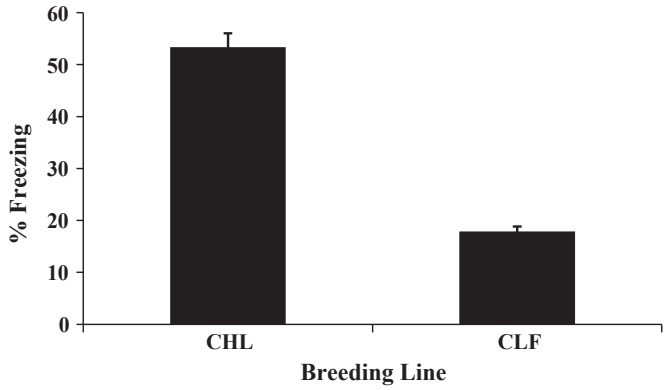


**Fig. 1.** Mean ( ± SEM) percentage of freezing in 17th-generation CHF and CLF rats ($n=10$ per group) during the 8 min contextual fear conditioning test.

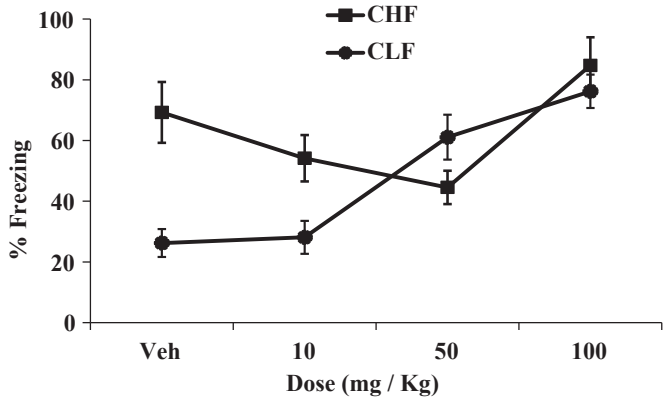


**Fig. 2.** Mean ( ± SEM) percentage of freezing in CHF and CLF animals ($n=10$ per group) injected with either vehicle or $(PhSe)_2$ at doses of 10, 50,and 100 mg/kg.

freezing in CHF and CLF rats after an i.p. injection of vehicle or $(PhSe)_2$ at doses of 10, 50, or 100 mg/kg. The results were statistically analyzed using two-way analysis of variance (ANOVA). The first factor, with two levels, was related to the breeding line (CHF and CLF). The second factor, with four levels, was related to the $(PhSe)_2$ dose (vehicle, 10, 50, or 100 mg/kg). The analysis revealed a main effect of breeding line ($F_{1,72}=9.05$, $p<0.005$) and $(PhSe)_2$ dose ($F_{3,72}=11.61$, $p<0.001$). An interaction between breeding line and $(PhSe)_2$ dose ($F_{3,72}=6.31$, $p<0.01$) was also detected. Pairwise *post hoc* comparisons indicated that CHF animals that received 50 mg/kg $(PhSe)_2$ exhibited less reliable conditioned freezing compared with CHF animals that received vehicle ($p<0.05$). The CHF animals that received 50 mg/kg $(PhSe)_2$ exhibited consistently more conditioned freezing compared with CLF animals that also received 50 mg/kg $(PhSe)_2$($p<0.05$). Pairwise *post hoc* comparisons indicated that CLF animals that received either 50 or 100 mg/kg $(PhSe)_2$ exhibited significantly more conditioned freezing compared with CLF animals that received vehicle (both $p<0.01$).

## 4. Discussion

In the present study, $(PhSe)_2$ was administered at three doses (10, 50, and 100 mg/kg) 30 min before measuring freezing patterns in CHF and CLF animals. The choice of dose and time was based on previous reports that showed that the maximal pharmacological effects of $(PhSe)_2$ can be achieved after 30 min in depression-like, inflammation, and antinociception models (Savegnago et al., 2007a, 2007b). Marina et al. provided baseline pharmacokinetic data for $(PhSe)_2$ and its absorption profile in plasma. They showed that the maximum concentration ($C_{max}$) was achieved 30 min after oral administration. The $C_{max}$ could be responsible for the observed pharmacological and toxicological effects of $(PhSe)_2$in acute experimental models (Prigol et al., 2009).

Interestingly, both dose- and line-dependent effects were observed for $(PhSe)_2$. Fig. 2 shows that $(PhSe)_2$ at 10 and 50 mg/kg reduced freezing (i.e., an anxiolytic-like effect) in CHF rats. Our results are consistent with Savegnago et al. (2007a, 2007b),who showed that $(PhSe)_2$ produced significant antidepressant- and anxiolytic-like effects in mice 30 min after oral administration. Systemic $(PhSe)_2$ administration exerted an anxiolytic-like effect in rats, with no adverse effects on locomotion or exploratory activity (Nogueira and Rocha, 2011). Regarding the possible mechanism of the anxiolytic-like effect of $(PhSe)_2$, anxiety is very complex and has no known definitive neurobiological or neurochemical mechanism. However, the dysregulation of multiple receptor systems, such as γ-aminobutyric acid (GABA), *N*-methyl-D-aspartate, dopamine $D_2$ receptors, and 5-hydroxytryptamine (5-HT; serotonin), are reported to be responsible for the incidence or progression of anxiety-related disorders (Porter et al., 1989; Malizia et al., 1998; Goddard et al., 2001). Previous studies indicated that $(PhSe)_2$ can interact with or modulate different neuronal and neurotransmitter systems, which may account for its possible anxiolytic-like effects. The interactions between $(PhSe)_2$ and other organoselenides and GABAergic, serotonergic (5-$HT_{1A}$, $5HT_{2A}$, and 5-$HT_{2A/2C}$), noradrenergic ($\alpha_1$ and $\alpha_2$), and dopaminergic ($D_1$, $D_2$, and $D_3$) systems have been reported in detail. Depending on the structure of organochalcogens and route of administration,the possible inhibition of the L-arginine-nitric oxide-cyclic guanosine monophosphate pathway and modulation of peroxisome proliferator-activated receptorγ cannot be excluded in the observed psychological effects of selenium compounds (Nogueira and Rocha, 2011).

Our data indicate that at a higher dose (100 mg/kg), the compound exerted significant anxiogenic-like effects in both CHF and CLF rats. Data regarding the neurotoxic effects of organochalcogens have been scarcely reported in the literature. Chronic exposure to higher doses of $(PhSe)_2$ caused central effects in the brain, including seizures, in rodents (Nogueira and Rocha, 2011). Modulation of the glutamatergic and GABAergic systems (Nogueira and Rocha, 2011) has been reported to be partially responsible for its neurotoxic effects, in which seizures induced by higher doses of $(PhSe)_2$ were prevented by diazepam, phenobarbital, and muscimol (Nogueira and Rocha, 2011).

In strong contrast to the CHF line, $(PhSe)_2$ (even at lower doses) increased freezing behavior in CLF animals, thus demonstrating both anxiolytic- and anxiogenic-like effects of this organoselenium compound in a bidirectional line. Previous studies have described various toxic effects of $(PhSe)_2$, including the generation of free radicals, oxidation of simple mono- or di-thiols, and inhibition of various thiol-containing enzymes (Nogueira and Rocha, 2010). However, no clear evidence has been provided in the literature about the possible anxiogenic-like activity of $(PhSe)_2$ at low doses.

Importantly, the present study was conducted in a bi-directional line that is being bredto modify the expression of genes that underlie a particular phenotype. With regard to the genetic regulation of anxiety and related disorders, Hovatta et al. (2005) reported that the expression of the glutathione reductase (*GSR1*) and glyoxalase (*Glo1*) genes that are involved in antioxidative metabolism are highly correlated with anxiety-related phenotypes. The expression of these enzymes was higher in the most “anxious” mice and lower in the least “anxious” strains. However, several contradictory reports have also been published. To further explore the mechanism of anxiety regulation, Hambsch et al. (2010) and Distler et al. (2012) reported that methylglyoxal (MG), the substrate for GLO1, is responsible for the regulation of anxiety. The neuroendocrine, neurochemical, and neurogenetic profiles of the

CHF and CLF lines have not yet been explored. The results of such studies could lead to possible diagnostic and therapeutic interventions. The possible role of GLO1 and MG in the CHF and CLF phenotypes should be considered. The distinct genetic profiles of the CHF and CLF lines may influence biochemical function, which may lead to differential responses to different aversive situations and various drugs. CHF and CLF animals exhibit different genetic and neuronal vulnerability to different stressful situations, reflected by differences in freezing responses, and may respond differently to the same drug at different doses.

In conclusion, the present results indicated quiet different modulation of freezing behavior in CHF and CLF rat lines by DPDS. Infact, DPDS increased significantly the freezing in CLF and tended to decrease in CHF rats. The exact mechanism of the present findings is yet to be explored but involvement of the monoaminergic, noradrenergic, dopaminergic, serotonergic, GABAA and 5-HT receptors cannot be neglected under the possible pathways or mechanism involved in the apparent activities of DPDS.

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## References

Andersen, J.K., 2004. Oxidative stress in neurodegeneration: cause or consequence? Nat. Rev. Neurosci. 5, S18–S25.
Atmaca, M., Tezcan, E., Kuloglu, M., Ustundag, B., Tunckol, H., 2004. Antioxidant enzyme and malondialdehyde values in social phobia before and after citalopram treatment. Eur. Arch. Psychiatry Clin. Neurosci. 245, 231–235.
Augustyniak, A., Bartosz, G., Cipak, A., Duburs, G., Horáková, L., Luczaj, W., Majekova, M., Odysseos, A.D., Rackova, L., Skrzydlewska, E., Stefek, M., Strosová, M., Tirzitis, G., Venskutonis, P.R., Viskupicova, J., Vraka, P.S., Zarković, N., 2010. Natural and synthetic antioxidants: an updated overview. Free Radic. Res. 44, 1216–1262.
Benton, D., Cook, R., 1991. The impact of selenium supplementation on mood. Biol. Psychiatry 29, 1092–1098.
Benton, D., 2002. Selenium intake, mood and other aspects of psychological functioning. Nutr. Neurosci. 5, 363–374.
Bouayed, J., Kalinin, V.V., 2011. Relationship between oxidative stress and anxiety: emerging role of antioxidants within therapeutic or preventive approaches. In: Kalinin, V. (Ed.), Anxiety Disorders. InTech, Rijeka, pp. 27–35.
Castro-Gomes, V., Landeira-Fernandez, J., 2008. Amygdaloid lesions produced similar contextual fear conditioning disruption in the Carioca high- and low-conditioned freezing rats. Brain Res. 1233, 137–145.
Dias, G.P., Bevilaqua, M.C., Silveira, A.C., Landeira-Fernandez, J., Gardino, P.F., 2009. Behavioral profile and dorsal hippocampal cells in Carioca high-conditioned freezing rats. Behav. Brain Res. 205, 342–348.
Distler, M.G., Plant, L.D., Sokoloff, G., Hawk, A.J., Aneas, I., Wuenschell, G.E., Termini, J., Meredith, S.C., Nobrega, M.A., Palmer, A.A., 2012. Glyoxalase 1 increases anxiety by reducing $GABA_A$ receptor agonist methylglyoxal. J. Clin. Invest. 122, 2306–2315.
Goddard, A.W., Mason, G.F., Almai, A., Rothman, D.L., Behar, K.L., Petroff, O.A., Charney, D.S., Krystal, J.H., 2001. Reductions in occipital cortex GABA levels in panic disorder detected with $^1$H-magnetic resonance spectroscopy. Arch. Gen. Psychiatry 58, 556–561.
Hambsch, B., Chen, B.G., Brenndörfer, J., Meyer, M., Avrabos, C., Maccarrone, G., Liu, R.H., Eder, M., Turck, C.W., Landgraf, R., 2010. Methylglyoxal-mediated anxiolysis involves increased protein modification and elevated expression of glyoxalase 1 in the brain. J. Neurochem. 113, 1240–1251.
Hassan, W., Gomes, V.C., Piton, S., Batista Teixeira da Rocha, J., Landeira-Fernandez, J., 2013. Association between oxidative stress and contextual fear conditioning in Carioca high- and low-conditioned freezing rats. Brain Res. 1512, 60–67.
Hovatta, I., Tennant, R.S., Helton, R., Marr, R.A., Singer, O., Redwine, J.M., Ellison, J.A., Schadt, E.E., Verma, I.M., Lockhart, D.J., Barlow, C., 2005. Glyoxalase 1 and glutathione reductase 1 regulate anxiety in mice. Nature 438, 662–666.
Malizia, A.L., Cunningham, V.J., Bell, C.J., Liddle, P.F., Jones, T., Nutt, D.J., 1998. Decreased brain $GABA_A$-benzodiazepine receptor binding in panic disorder: preliminary results from a quantitative PET study. Arch. Gen. Psychiatry 55, 715–720.
Masood, A., Nadeem, A., Mustafa, S.J., O'Donnell, J.M., 2008. Reversal of oxidative stress-induced anxiety by inhibition of phosphodiesterase-2 in mice. J. Pharmacol. Exp. Ther. 326, 369–379.
Nogueira, C., Rocha, J.B.T., 2011. Toxicology and pharmacology of selenium: emphasis on synthetic organoselenium compounds. Arch. Toxicol. 85, 1313–1359.
Nogueira, C.W., 2010. Rocha JBT. Diphenyl diselenide a janus-faced molecule. J. Braz. Chem. Soc. 21, 2055–2071.
Nogueira, C.W., Zeni, G., Rocha, J.B., 2004. Organoselenium and organotellurium compounds: toxicology and pharmacology. Chem. Rev. 104, 6255–6285.
Paulmier, C., 1986. Selenoorganic functional groups. In: Paulmier, C. (Ed.), Selenium Reagents and Intermediates in Organic Synthesis. Pergamon Press, Oxford, pp. 25–51.
Porter, J.H., Wiley, J.L., Balster, R.L., 1989. Effects of phencyclidine-like drugs on punished behavior in rats. J. Pharmacol. Exp. Ther. 248, 997–1002.
Prigol, M., Schumacher, R.F., Nogueira, C.W., Zeni, G., 2009. Convulsant effect of diphenyl diselenide in rats and mice and its relationship to plasma levels. Toxicol. Lett. 189, 35–39.
Rayman, M.P., 2000. The importance of selenium to human health. Lancet 356, 233–241.
Salim, S., 2011. Oxidative stress in anxiety: implications for pharmacotherapy. Am. J. Integr. Med. 1, 11–21.
Savegnago, L., Jesse, C.R., Pinto, L.G., Rocha, J.B.T., Nogueira, C.W., 2007a. Diphenyl diselenide attenuates acute thermal hyperalgesia and persistent inflammatory and neuropathic pain behavior in mice. Brain Res. 1175, 54–59.
Savegnago, L., Pinto, L.G., Jesse, C.R., Alves, D., Rocha, J.B.T., Nogueira, C.W., Zeni, G., 2007b. Antinociceptive properties of diphenyl diselenide: evidence for the mechanism of action. Eur. J. Pharmacol. 555, 129–138.
Sher, L., 2000. Selenium and human health. Lancet 356, 943.
Sher, L., 2007. Possible role of selenium deficiency in the neurobiology of depression and suicidal behavior in patients with alcohol use disorders. Int Disabil. Human Dev. 6, 227–230.