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Review

The test retest model of anxiety: An appraisal of findings to explain benzodiazepine tolerance

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ABSTRACT

A test retest protocol in animal model of anxiety induces an increase of anxious behavior and a loss of benzodiazepine-induced effect. This effect, known as the "one trial tolerance", is mainly observed in the elevated plus maze, an ethological model of anxiety in mice, but also in the four plate test, a model based on punishment. A review of some hypotheses based on behavioral, pharmacological and neurochemical approaches are proposed here to explain this benzodiazepines tolerance phenomenon.

Fear is a normal reaction to a threatening situation, frequently observed in everyday life. The fear can be used as adaptive mechanism of alarm for the organism; however it can also induce damaging effects when the anxious feeling persists. Benzodiazepines (BDZs) still remain the main acutely effective anxiolytic agents, and because of their extensive use, the development of animal model of anxiety has been based on their pharmacology (Bourin, 1997). However, there are non-genetic non-pharmacological manipulations that lead to modulate the general stress levels of animals, which when performed before testing, have profound effects on behavior including manipulations such as chronic restraint or chronic unpredictable mild stress (Zhu et al., 2014), photoperiod changes (Tal-Krivisky et al., 2015), exposure to predator odor (Matar et al., 2013) and more. Behavior can be both an event and a process and observable behaviors are the result of the integration of all of the processes ongoing in underlying organ systems, in interaction with the external social and physical environment (Bourin, 2015).

Surprisingly, researchers have found a reduction in the efficiency of BZDs if used on experienced mice in some animal models of anxiety. This phenomenon, called one-trial tolerance (OTT) to BZD substances, has been initially described as a marked attenuation, or even an abolition of the response to an anxiolytic compound, induced by a previous single undrugged experience of the elevated plus maze (EPM) (File, 1990; Lister, 1987). This abolition of the response of BZDs was called OTT in contrast to the normal 3-week treatment of BZD that is necessary to obtain tolerance to anxiolytic effect in the test (File et al., 1992; File, 1993). Same results have been obtained when administering barbiturates, alcohol, serotonin 1_A receptor agonists, and glutamatergic antagonists (Bertoglio and Carobrez, 2002, 2009; Gomes and Nunes-De-Souza, 2003). Mainly reported in EPM, loss of BZD effects has been found in other animal models of anxiety, e.g. light/dark transition (Holmes et al., 2001), predator-odor-exposure test (McGregor and Dielenberg, 1999) and the four-plate test (FPT; Hascoët et al., 1997, Ripoll et al., 2005, 2006, Petit-Demoulière et al., 2008, Petit-Demoulière and Bourin, 2007, Petit-Demoulière et al., 2009).

There is now a substantive literature indicating that a single prior un-drugged exposure to the maze usually results in increased open arm avoidance on subsequent trials; perhaps indicating increased anxiety. The anxiolytic efficacy of BDZs is either markedly reduced or completely abolished by prior undrugged test experience.

Several hypotheses based on behavioral, pharmacological and neurochemical approaches have been proposed to explain the benzodiazepines tolerance phenomenon in the EPM.

1. Baseline anxiety: To explain OTT, some authors suggest that prior exposure to the test could increase baseline anxiety leading to the elimination of the anxiolytic-like effect of BZD (Cruz-Morales et al., 2002; File et al., 1992; Holmes and Rodgers, 1999).
2. Locomotion: The team of Dawson (Dawson et al., 1994; Dawson and Tricklebank, 1995) was the first who suggested that the loss of BZD activity during the second trial came from a familiarizing effect of animal to the test that leads to a decrease in locomotor activity. However, this hypothesis was rejected as the number of closed arms entries in an EPM, a parameter considered to indicate locomotor activity, was unchanged during the second trial (Rodgers and Johnson, 1995).
3. Emotional state: An additional hypothesis explains the tolerance to benzodiazepines as a change in emotional state (File et al., 1993, Holmes and Rodgers, 1999). It suggests that mice developed anticipatory anxiety reaction and that previous exposures to the test resulted in a phobic state against which benzodiazepines are

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ineffective (File et al., 1993).

4. Lack of controversial motivation: Rodgers and Shepherd (1993) suggested that the tolerance to the BZDs during a second exposition might be explained by the lack of conflict between exploration and fear of open arms. Indeed, the familiarity of the different parts of the EPM by the animal results in a decrease in the exploration of the aversive areas. This hypothesis was supported by the study of Pereira et al. (1999) that observed that when an additional aversive parameter was introduced during the second trial (hot breath of air and light in the closed arms) the anxiolytic effect of BZD was restored. The fact that animal can choose between different aversive situations might be implicated in the training process (Bertoglio and Carobrez, 2000).
5. Change in the GABAA complex receptor: Gonzalez and File (1997) suggested that during the first trial, an endogenous inverse agonist is released, binds to, and desensitizes the benzodiazepine receptors. The presence of this changed state of benzodiazepine receptors is manifested during retesting. Some studies also suggested that the first maze experience modified the whole GABAA complex receptor and not only the BZD receptors (Bertoglio and Carobrez, 2002). Indeed the one trial tolerance had been observed for other compounds binding to the $GABA_A$ receptor such as ethanol and phenobarbital (Bertoglio and Carobrez, 2002, Cole et al., 1999, File, 1993). However, this hypothesis has to be confirmed and some controversial results were shown.
6. Learning: learning had been studied in this context in order to evaluate the influence of memory in OTT. It had been shown that the use of amnesic agents such as scopolamine did not modify the OTT observed when administering chlordiazepoxide in the plus maze (Calzavara et al., 2005). Atropine sulphate, a muscarinic cholinergic receptor antagonist known for its amnesic properties, did not significantly raise the number of punished crossings in retest mice in the four plate test (FPT; Ripoll et al., 2005). In contrast, other studies concluded that OTT implies an aversive learning within trial 1 that is transferred to trial 2 (Vargas et al., 2006).

In our laboratory, we performed test-retest using the FPT and demonstrated that benzodiazepine diazepam lost its efficacy on the retest (Bourin et al., 2007). The FPT in mice (Aron et al., 1971, Boissier et al., 1968) is based on exploration of novel surrounding suppressed by the delivery of mild electric foot shock contingent to quadrant crossing. This model had been demonstrated to be sensitive to BZDs. During a second exposure to the four plates test, mice demonstrated a dramatic decrease in exploratory activity, with an inhibition of the punished crossings. Furthermore, mice tried to escape from the box. This effect appeared as soon as mice were placed in the test box, even before receiving any electric shocks. This decrease in activity was persistent even with a 42 days interval between the two sessions. The use of the FPT test-retest had been the first step for a better characterization of OTT. Different ways of investigation had been used in order to understand OTT and aversive memory in the FPT test-retest protocol, i.e., spatial and temporal parameters modifications, pharmacological manipulations and brain structural manipulation.

In one study (Petit-Demoulière and Bourin, 2007), Swiss mice were subjected to the modified FPT procedure. Electric punishments were removed on the first trial, so the stressful situation was only linked to the unknown apparatus. FPT was then conducted without punishments in both Trials. FPT with spatial modifications was used. In the last experiment, an EPM was performed instead of the FPT in the trial 1. Removing punishments in trial 1 did not counteract the reduction effect of diazepam, but spatial modifications of the aversive environment did so in trial 2. Exposure to EPM did not trigger a loss of efficacy of diazepam. We found that electric punishments were not responsible for the one-trial tolerance to BZD, but were implicated in aversive memory. However the electric foot shocks acted as a reinforcing stimulus. Spatial knowledge had been studied in the FPT and in the EPM. Complete spatial representation of the FPT seems to be responsible for the OTT. In the EPM, the experience of open arm, or closed arm, isolation during trial 1 had been related to the loss of BZD efficacy on retest (File et al., 1990, 1994; Holmes and Rodgers, 1999). From that study came the terminology of OTT as the loss of BZDs efficacy during the second trial, and aversive learning with a strong decrease in activity of undragged experienced mice as compared with naïve mice during the second trial. Temporal parameters are strongly implicated in the emergence of this phenomenon. Indeed, aversive memory and OTT appear after a brief exposure to FPT, respectively 45 s. and 30 s (Petit-Demoulière and Bourin, 2007). In EPM, one-trial tolerance was dependent on the length of trial 1 (Dal-Cól et al., 2003, File et al., 1993) and both trials and appeared after the second minute of Trial 1 (Calzavara et al., 2005). It total, these data suggests that spatial and temporal factors are critical for the OTT phenomenon.

One of our major finding showed that the $5\text{-HT2}_{A/2C}$ agonist DOI prevented the increase in anxiety during retest through 5-HT_{2A} subtype receptor (Ripoll et al., 2006). DOI was previously reported to induce anxiolytic-like effect in naive mice from 0.5 to 4 mg/kg (Nic Dhonnchadha et al., 2003). In experienced mice, DOI produced its anti-punishment effect from 1 to 4 mg/kg in a similar manner. Among the three antagonists studied in association with DOI, SR 46349B, SB 206553 and RS 10-2221, respectively, 5-HT_{2A}, $5\text{-HT}_{2B/2C}$ and 5-HT_{2C} receptor antagonists, deprived of effect alone, only the SR 46349B was able to antagonize the DOI-induced effect demonstrating that DOI exerts its anxiolytic-like effect through 5-HT_{2A} receptors. The exact mechanism remains to be studied. 5-HT_{2A} receptor subtypes play a dual role on the GABAergic system in the CNS. On the one hand, studies demonstrated that at presynaptic level, 5-HT_{2A} receptors exert an excitatory influence upon GABAergic neurones in the amygdala (Sokal et al., 2005), the hippocampus (Shen and Andrade, 1998), the PAG (Griffitsh and Lovick, 2002) suggested mediating the anxiolytic action of 5-HT_{2A} agonists. On the other hand, 5-HT_{2A} receptors stimulation at post-synaptic level had been shown to suppress GABAergic transmission in prefrontal cortex (Yan, 2002). Depending on the anxiety models, 5-HT_{2A} ligands may specifically exert facilitator (pre-synaptic) or inhibitory (post-synaptic) influence upon GABAergic transmission through 5-HT_{2A} receptors explaining their contrasting roles in the modulation of anxious states (Millan, 2003). However, whether the hypothetic excitatory influence upon GABAergic is directly (through 5-HT_{2A} located on GABAergic) or indirectly triggered is still to be determined. An additional level of complication is that in fact, 5-HT_{2A} receptors are located not only on serotonergic and GABAergic but also on noradrenergic and dopaminergic neurons (Nocjar et al., 2002).

The last step in understanding the test retest paradigm, concern brain structures implicated the OTT and aversive learning. In a recent study, brain local injections were used in order to localize structures implied in naïve and experienced mice. Periacqueductal grey (PAG) substance, three sub-regions of hippocampus (CA1, CA2 and CA3) and two nuclei of amygdala (BLA and LA) were studied. Local injections did not cause any modifications of ambulatory activity. Brain structures were functionally differently involved. DOI injections elicit anxiolytic-like effects only when injected into CA2, in naïve and experienced mice. Diazepam had an anxiolytic-like effect in naïve mice, only when injected into the lateral nucleus of amygdala, and in experienced mice when injected into PAG. These results supported the hypothesis that the mechanism underlying anxiety in naïve and experienced mice might be different.

The kind of anxiety that occurred in experienced mice differed from the anxiety that occurred in unexperienced mice, implicating functional and structural changes in neuronal pathways. The kind of anxiety implicated in the test- retest FPT and EPM is not fully understood. Interestingly, no “one trial tolerance” was found in the punished drinking test. Diazepam still demonstrated anxiolytic effects whatever the number of exposures to the test (File et al., 1993). The punished drinking test is one of the conflict tests that requires previous training

procedure similar to the Geller-Seifter conflict test. Those procedures did not induce reduced efficacy of benzodiazepine treatment.

The OTT phenomenon might be directly related to the clinical environment. First, OTT could explain why BZDs are ineffective for PTSD treatment and prevention, and why the risks associated with their use tend to outweigh potential short-term benefits (Guina et al., 2015). In fact, extrapolating from the OTT, is can be suggested that PTSD is more related to fear than to anxiety. Yet, the mechanisms responsible for these phenomena are not yet known. Benzodiazepines act via GABA(A) receptors, but the short OTT exposure suggests that the tolerance is not due to simple downregulation of receptor number (Bateson, 2002).

The OTT and aversive learning found in the test- retest FPT and EPM is a complex phenomenon not fully understood. However, some new directions in research emerged from recent findings. The implication of the PAG in experienced mice behavior suggested the involvement of the brain defense system during the second trial. The behavior of experience mice in the second trial of a test retest paradigm might be related to fear rather than to anxiety.

References

Aron, C., Simon, P., Larousse, C., Boissier, J.R., 1971. Evaluation of a rapid technique for detecting minor tranquilizers. Neuropharmacology 10, 459–469.

Bateson, A.N., 2002. Basic pharmacologic mechanisms involved in benzodiazepine tolerance and withdrawal. Curr. Pharm. Des. 8, 5–21.

Bertoglio, L.J., Carobrez, A.P., 2000. Previous maze experience required to increase open arms avoidance in rats submitted to the elevated plus maze model of anxiety. Behav. Brain Res. 108, 197–203.

Bertoglio, L.J., Carobrez, A.P., 2002. Anxiolytic effects of ethanol and phenobarbital are abolished in test-experienced rats submitted to the elevated plus maze. Pharmacol. Biochem. Behav. 73, 963–969.

Bertoglio, L.J., Carobrez, A.P., 2009. Anxiolytic-like effects of NMDA/glycine-B receptor ligands are abolished during the elevated plus-maze trial 2 in rats. Psychopharmacology 170, 335–342.

Boissier, J.R., Simon, P., Aron, C., 1968. A new method for rapid screening of minor tranquillizers in mice. Eur. J. Pharmacol. 4, 145–151.

Bourin, M., 1997. Animal models of anxiety: are they suitable for predicting drug action in humans? Pol. J. Pharmacol. 49, 79–84.

Bourin, M., 2015. Animal models for screening anxiolytic-like drugs: a perspective. Dialogues Clin. Neurosci. 3, 295–303.

Bourin, M., Petit-Demoulière, B., Dhonnchadha, B.N., Hascöet, M., 2007. Animal models of anxiety in mice. Fundam. Clin. Pharmacol. 6, 567–574.

Calzavara, M.B., Patti, C.L., Lopez, G.B., Abilio, V.C., Silva, R.H., Frussa-Filho, R., 2005. Role of learning of open arm avoidance in the phenomenon of one-trial tolerance to the anxiolytic effect of chlordiazepoxide in mice. Life Sci. 76, 2235–2246.

Cole, R.P., Oberling, P., Miller, RR., 1999. Recovery from one-trial overshadowing. Psychon. Bull. Rev. 6 (3), 424–431 (Sep).

Cruz-Morales, S.E., Santos, N.R., Brandao, M.L., 2002. One-trial tolerance to midazolam is due to enhancement of fear and reduction of anxiolytic-sensitive behaviors in the elevated plus- maze retest in the rat. Pharmacol. Biochem. Behav. 72, 973–978.

Dal-Cól, M.L., Pereira, L.O., Rosa, V.P., Calixto, A.V., Carobrez, A.P., Faria, M.S., 2003. Lack of midazolam-induced anxiolysis in the plus-maze Trial 2 is dependent on the length of Trial 1. Pharmacol. Biochem. Behav. 74 (2), 395–400 (Jan).

Dawson, G.R., Tricklebank, M.D., 1995. Use of the elevated plus maze in the search for novel anxiolytic agents. Trends Pharmacol. Sci. 16, 33–36.

Dawson, G.R., Crawford, S.P., Stanhope, K.J., Iversen, S.D., Tricklebank, M.D., 1994. One-trial tolerance to the effects of chlordiazepoxide on the elevated plus maze may be due to locomotor habituation, not repeated drug exposure. Psychopharmacology 113, 570–572.

File, S.E., 1990. One-trial tolerance to the anxiolytic effects of chlordiazepoxide in the plus-maze. Psychopharmacology 100, 281–282.

File, S.E., 1993. The interplay of learning and anxiety in the elevated plus-maze. Behav. Brain Res. 58, 199–202.

File, S.E., Mabbutt, P.S., Hitchcott, P.K., 1990. Characterisation of the phenomenon of "one-trial tolerance" to the anxiolytic effect of chlordiazepoxide in the elevated plus-maze. Psychopharmacology 102, 98–101.

File, S.E., Andrews, N., Wu, P.Y., Zharkovsky, A., Zangrossi Jr., H., 1992. Modification of chlordiazepoxide's behavioural and neuro- chemical effects by handling and plus-maze experience. Eur. J. Pharmacol. 218, 9–14.

File, S.E., Zangrossi Jr., H., Viana, M., Graeff, F.G., 1993. Trial 2 in the elevated plus-maze: a different form of fear? Psychopharmacology 111, 491–494.

File, S.E., Zangrossi Jr., H., Sanders, F.L., Mabbutt, P.S., 1994. Raised corticosterone in the rat after exposure to the elevated plus-maze. Psychopharmacology 113, 543–546.

Gomes, K.S., Nunes-De-Souza, R.L., 2003. Implication of the 5-HT2A and 5-HT2C (but not 5HT1A) receptors located within the periaqueductal gray in the elevated plus-maze test-retest paradigm in mice. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 33, 1261–1269.

Gonzalez, L.E., File, S.E., 1997. A five minute experience in the elevated plus maze alters the state of benzodiazepine receptor in the dorsal raphe nucleus. J. Neurosci. 17, 1505–1511.

Griffitsh, J.L., Lovick, T.A., 2002. Co-localization of 5-HT2A receptor and GABA-immunoreactivity in neurons in the periaqueductal grey matter of the rat. Neurosci. Lett. 326, 151–154.

Guina, J., Rossetter, S.R., De Rhodes, B.J., Nahhas, R.W., Welton, R.S., 2015. Benzodiazepines for PTSD: a systematic review and meta-analysis. J. Psychiatr. Pract. 21, 281–303.

Hascoët, M., Bourin, M., Couetoux du Tertre, A., 1997. Influence of prior experience on mice behavior using the four-plate test. Pharmacol. Biochem. Behav. 58, 1131–1138.

Holmes, A., Rodgers, R.J., 1999. Influence of spatial and temporal manipulations on the anxiolytic efficacy of chlordiazepoxide in mice previously exposed to the elevated plus-maze. Neurosci. Biobehav. Rev. 23, 971–980.

Holmes, A., Iles, J.P., Mayell, S.J., Rodgers, R.J., 2001. Prior test experience compromises the anxiolytic efficacy of chlordiaz-epoxide in the mouse light/dark exploration test. Behav. Brain Res. 122, 159–167.

Lister, R.G., 1987. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology 92, 180–185.

Matar, M.A., Zohar, J., Cohen, H., 2013. Translationally relevant modeling of PTSD in rodents. Cell Tissue Res. 354, 127–139.

McGregor, I.S., Dielenberg, R.A., 1999. Differential anxiolytic efficacy of a benzodiazepine on first versus second exposure to a predatory odor in rats. Psychopharmacology 147, 174–181.

Millan, M.J., 2003. The neurobiology and control of anxious states. Prog. Neurobiol. 22, 675–688.

Nic Dhonnchadha, B.A., Bourin, M., Hascoet, M., 2003. Anxiolytic-like effects of 5-HT2 ligands on three mouse models of anxiety. Behav. Brain Res. 140, 203–214.

Nocjar, C., Roth, B.L., Pehek, E.A., 2002. Localization of 5-HT(2A) receptors on dopamine cells in subnuclei of the midbrain A10 cell group. Neuroscience 111, 163–176.

Pereira, J.K., Vieira, R.J., Konishi, C.T., Ribeiro, R.A., Frussa-Filho, R., 1999. The phenomenon of "one-trial tolerance" to the anxiolytic effect of chlordiazepoxide in the elevated plus-maze is abolished by the introduction of a motivational conflict situation. Life Sci. 65, PL101–PL107.

Petit-Demoulière, B., Bourin, M., 2007. Temporal parameters of one-trial tolerance to benzodiazepines in four-plate test-retest. Behav. Brain Res. 183, 222–225.

Petit-Demoulière, B., Hascoët, M., Bourin, M., 2008. Factors triggering abolishment of benzodiazepines effects in the four-plate test-retest. Eur. Neuropsychopharmacol. 18, 41–47.

Petit-Demoulière, B., Massé, F., Cogrel, N., Hascoët, M., Bourin, M., 2009. Brain structures implicated in the four-plate test in naïve and experienced Swiss mice using injection of diazepam and the 5-HT2A agonist DOI. Behav. Brain Res. 204, 200–205.

Ripoll, N., Nic Dhonnchadha, B.A., Sebille, V., Bourin, M., Hascoët, M., 2005. The four-plates test–retest paradigm to discriminate anxiolytic effects. Psychopharmacology 180, 73–83.

Ripoll, N., Hascoët, M., Bourin, M., 2006. Implication of 5-HT2A subtype receptors in DOI activity in the four-plates test-retest paradigm in mice. Behav. Brain Res. 166, 131–139.

Rodgers, R.J., Johnson, N.J., 1995. Factor analysis of spatiotemporal and ethological measures in the murine elevated plus maze of anxiety. Pharmacol. Biochem. Behav. 52, 297–303.

Rodgers, R.J., Shepherd, J.K., 1993. Influence of prior maze experience on behavior and response to diazepam in the elevated plus maze and light/dark tests of anxiety in mice. Psychopharmacology 113, 237–242.

Shen, R.Y., Andrade, R., 1998. 5-Hydroxytryptamine2 receptor facilitates GABAergic neurotransmission in rat hippocampus. J. Pharmacol. Exp. Ther. 285, 805–812.

Sokal, D.M., Giarola, A.S., Large, C.H., 2005. Effects of GABA(B), 5-HT(1A),and 5-HT(2) receptor stimulation on activation and inhibition of the rat lateral amygdala following medial geniculate nucleus stimulation in vivo. Brain Res. 1031, 141–150.

Tal-Krivisky, K., Kronfeld-Schor, N., Einat, H., 2015. Voluntary exercise enhances activity rhythms and ameliorates anxiety- and depression-like behaviors in the sand rat model of circadian rhythm-related mood changes. Physiol. Behav. 151, 441–447.

Vargas, K.M., Da Cunha, C., Andreatini, R., 2006. Amphetamine and pentylenetetrazole given post-trial 1 enhance one-trial tolerance to the anxiolytic effect of diazepam in the elevated plus-maze in mice. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 30, 1394–1402.

Yan, Z., 2002. Regulation of GABAergic inhibition by serotonin signaling in prefrontal cortex: molecular mechanisms and functional implications. Mol. Neurobiol. 26, 203–216.

Zhu, S., Shi, R., Wang, J., Wang, J.F., Li, X.M., 2014. Unpredictable chronic mild stress not chronic restraint stress induces depressive behaviours in mice. Neuroreport 25 (14), 1151–1155.